Colour Polymorphism in the Common Buzzard: Evolution and Life History Consequences

Martin Kenneth Fowlie

UNIVERSITY
of
GLASGOW

This thesis is submitted for the degree of Doctor of Philosophy

Division of Environmental & Evolutionary Biology
Institute of Biomedical & Life Sciences
University of Glasgow

October 2003

CANDIDATE'S DECLARATION

I declare that the work recorded in this thesis is entirely my own unless otherwise stated and that it is of my own composition. No part of this work has been submitted for any other degree.

Martin Fowlie
October 2003

ACKNOWLEDGEMENTS

Firstly, I must thank my parents, who despite not really being interested themselves, made sure that I never lost my interest in natural history and ferried me about to various clubs and societies and stopped the car whenever I saw something interesting on holiday. They put up with my not getting a 'real job' and allowed to me follow my passion. My sister put up with her know-it-all brother and being taken to places of no interest to her. All three have always provided much support, both emotional and financial whenever it was most needed over many years.

My undergraduate at Sheffield was fairly unremarkable but my interest in evolution was maintained and strengthened by Ben Hatchwell and Mike Siva-Jothy. Working for Ben after my degree was a very enjoyable time and I learnt much about fieldwork and experimental design, and I still can't walk through a wood without wanting to run after Lottis every time I hear them!

Thanks also to Andrew Cockburn, Mike Double, Jan Komdeur, Terry Burke and Dave Richardson for letting me study interesting species in even more interesting places.

I have learnt a lot from people during my PhD and there are numerous people who have had an influence on my thinking. Stu Bearhop, Stu Humphries, Jon Blount, Hanna Kokko, Lukas Keller, Pat Monaghan, Neil Metcalfe, Fi McPhie, Kate Arnold have all provided useful feedback at various times. Thanks to Lorna Kennedy for the admin stuff.

The social life has been great and I must thank my lab mates, Mike Fowler, Lesley Morrell, Sarah Brown, Jo Mould, Andrew 'Lucky Charms' Jackson, Chris 'The Beast' Fraser, wee Fi 'three strikes and you're out' McPhie and Emma Ådahl. Trips to the pub have been more frequent that even I could have ever envisaged. Jen A, Jen W., Jan, Martin and Hayley, Steve Votier, Stu B., Stu H., Jon B., Nick Royle, Mike, Maria, Sarah, Jo, Lesley, Craig, Claire, Chris, Dave Stevens, Fi, Ben. Thanks to non-work mates who made me see there was a world outside of work: Niall, Gaynor, Euan, Bindi, Clare. If I've forgotten anyone I'm sorry and I'll fit you in the 2nd edition!

My time in Cambridge lasted much longer than I thought but was made bearable by a number of general good eggs. Sasha Dall, Becky Kilner, Emma Cunningham, Anne and Ron Carlson, Mandy Ridley, Sarah Hodge, Andy Young, Tim Coulson, Mike Cant,

Rufus Johnstone all offered their company in the local hostelries. Joe, Kanchon, Lilly, Kai, Alvaro, Oliver, Dan, Ed, Karina all made the molecular ecology lab a nice place to work. Thanks to Matt Wood for frequent 'chats' in Sheffield on my way back to Glasgow.

Andy Russell and Virpi Lummaa put me up for nigh on nine months and never got tired of me stumbling in at all times of the night. This was probably due to the fact that Andy usually stumbled in 10 seconds later. Thanks to the 'girls': Terho, Pilkku and Onni for always being glad to see me. A special thank you to Andyman for all his help and discussion about the wonderful world of biology and making me see that actually co-operative breeding in space might be the way to go.

Oliver Krüger kindly allowed me access to data and provided field support and extensive discussions on all thing buzzard. Thanks to Thomas Grünkorn for the tree climbing and to Herr and Frau Krüger for their hospitality.

My two supervisors have both helped greatly. Bill Amos let me spend lots of time using his facilities and always offered lots of enthusiasm and incredibly helpful insight during times when I was beginning to lose heart.

Jan Lindström has been the best supervisor I could have wished for. Steering me in the right direction while letting me get on with it and always leaving enough rope for me to frequently hang myself. Jani, Kiitos mielettömästi kaikesta avustasi ja kannustuksestasi menneiden kolmen vuoden aikana, olen oppinut kauhean paljon. Olen todella nauttinut kanssasi työskentelystä, ja olen nauttinut juopottelusta vieläkin enemmän.

Finally, I have to thank the most important person in getting me through it all, Marion. Her constant support, good food and general looking after of me while being amenable to me being away for protracted periods of time have helped more than even she knows. I am very grateful and will try and somehow repay the faith she has had in me. Je t'aime.

This work was funded by NERC studentship NERS/S/A/2000/03370 other financial support was gratefully received from ASAB.

STOP PRESS 6/11/03 Thanks to Erik Svensson and Graeme Ruxton for constructive criticism and discussion during the viva.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

SUMMARY

Understanding coevolution between phenotypic variation and other traits is of paramount importance in explaining the origin and maintenance of polymorphism in natural populations. We tested whether the apostatic selection hypothesis can explain the evolution of plumage polymorphism in raptors and owls. Results in neither of these bird groups supported this: plumage polymorphism was not more common in taxa hunting avian or mammalian prey, nor in migratory species. In contrast, we found that polymorphism was related to variables such as population size and range size, as well as breeding altitude and breeding latitude.

The Common Buzzard shows three plumage colour polymorphisms, which differ in their lifetime reproductive success. This species has previously been suggested to mate maladaptively with respect to which colour morph it chooses as a mate. We compared the observed mating pattern of mating with one's mother's phenotype by using a demographically structured population model as a basis for an evolutionary invasion analysis. The mating strategies competing with the observed one were random, mating with one's own phenotype, mating with an individual dissimilar to one's mother's phenotype and mating to maximise fitness. We showed that buzzards do indeed appear to mate maladaptively but only if we assume that the genes for mate choice "are aware of" which phenotype they reside in. If this is not the case, due to potential genetic constraints, buzzards do mate adaptively although the observed strategy appears to be 'the best of a bad job.'

The detrimental effects of inbreeding are well known, and they have been shown to be associated with lower levels of reproductive success, higher levels of parasitism and differences in disease susceptibility. To better understand large fitness differences between morphs in the colour polymorphic common buzzard, *Buteo buteo*, we investigated differences in the levels of internal relatedness between morphs. As the common buzzard mating system is non-random and the light and dark morph individuals are less abundant than the intermediates, it could be the case the extreme colour morph individuals are more inbred. However, no differences were found in levels of inbreeding.

In birds, the physiological and behavioural consequences of colour polymorphisms are not widely known. Here we used an experiment to investigate the effect of this melanin-based polymorphism on nest defence behaviour in the common buzzard. Among males, light morphs were found to be significantly more aggressive to a perceived threat of nest predation than either intermediate or darkly coloured birds, while there was a non-significant tendency for the reverse among females. The level of aggression observed for each member of a pair was independent of the level of aggression shown by the other member. These results illustrate that polymorphisms can be associated with alternative reproductive tactics in birds, and suggest a possible link between the biochemistry of melanin production and individual behaviour.

For most species living in seasonal environments, timing is an important determinant of the success of a breeding attempt. Individuals also face a trade off between current and future reproduction. Here we investigated whether colour morphs differed in their timing of breeding. Light-light and dark-dark pairs were found to breed earlier

than the population mean, with light-dark pairs fledging chicks slightly later. Differences in reproductive strategies between morphs may account for the observed differences.

Chapter 1

GENERAL INTRODUCTION

Colour Polymorphism

The Oxford dictionary definition of a polymorphism is: "the existence of two or more forms that are distinct from one another but contained within the same breeding population."

Evolutionary ecologists focussed on stable genetic polymorphisms and the processes that lead to and maintain them during the 1950s and 60s (Dobzhansky 1970). This led to theoretical predictions concerning the evolutionary dynamics and ecological mechanisms involved. As a noticeable phenomenon colour polymorphisms were an obvious choice for study. Colour polymorphism, the occurrence of two or more distinct colour phenotypes within a population, has been described in a wide range of taxa. For vertebrates all major taxa contain species, which exhibit this phenomenon (Fish, Horth 2003; Amphibians, Hoffman and Blouin 2000; Reptiles, Sinervo and Lively 1996; Birds, Theron et al. 2001; Mammals, Ritland et al. 2001).
In invertebrates there are well-documented cases in marine isopods (Merilaita 2001) and molluscs (Singh 1981; Cook 1990, 1992).

In humans, perhaps the best-known case is that of the balanced polymorphism for sickle cell anaemia (Allison 1955, 1964). In areas where malaria is prevalent, individuals that are heterozygous for the sickle cell gene are less susceptible to the disease than homozygote non-carriers, the other homozygote being lethal. This is a classic case of heterosis or heterozygote advantage.

Four main hypotheses have been proposed to explain the evolution of colour polymorphism in birds (Galeotti et al. 2003).

1. Apostatic selection

This is a type of frequency-dependent selection where it is advantageous for the mutant morph to be different from the majority of other morphs (Paulson 1973; Rohwer 1983; Rohwer & Paulson 1987). Prey are less 'familiar' with this morph and they do not have an 'avoidance image' for this rare morph. Selection will favour the rare morph until a balance between morphs is achieved, mediated by the prey. This theory is often proposed to explain polymorphism in raptors and skuas (Rohwer & Paulson 1987).

2. Disruptive selection

A balanced colour polymorphism may arise if disruptive selection favours the extreme individuals in a normally distributed population (Greene et al. 2000). Camouflage or crypsis in a heterogeneous habitat may result in different morphs being selected for (Galeotti & Cesaris 1996). The main group for which the mimetic function of colour polymorphism has been proposed is the herons (Murton, 1971; Caldwell, 1986). However, some authors find no relationship between morph and habitat background (Itoh, 1991).

3. Non-random mating

Fisher (1930) stressed, the existence of a permanent polymorphism implies a selective balance between the two (or more) alternative morphs, both enjoying some selective advantage but also suffering some disadvantage. Colour polymorphism may

arise from some preferences in mate-choice. Female choice for conspicuous males may evolve if bright colour is an honest signal coupled with higher predation pressure. Lower quality males are unable to produce this costly trait and become a more cryptic morph (Endler 1980, 1983, 1987).

Disassortative mating may also lead to polymorphism (Lowther 1961; Murton et al. 1973). One possible reason for disassortative mating is to aim at optimal outbreeding if genetically diverse offspring are favoured. Disassortative mating can maintain polymorphisms by favouring rare morphs, frequencies will then change over time.

4. No selection

Colour polymorphism may be a neutral non-adaptive trait genetically correlated to another ecological trait, i.e. pleiotropy. Alternatively, the colour polymorphism may be neutral *per se*. If this were true then it would be expressed in large predators, free from predation pressure, and there does not appear to be any good evidence for this.

These mechanisms are not mutually exclusive and may operate simultaneously in natural populations.

One of the best understood and much studied avian cases is the colour polymorphism in the lesser snow goose, *Chen caerulescens caerulescens* (Cooke *et al.* 1995). The existence of light and dark plumage morphs seems to be an historical phenomenon. The two morphs appear to have evolved in allopatry and contact between them was re-established in the recent past. Despite clear differences in colour, there appears to be no functional difference between morphs (Cooke *et al.* 1985). Young birds imprint on their parents' morph and this helps to maintain morph segregation by

positive assortative mating. Where mixed colonies occur and offspring are sometimes raised by parents of different morph, thereby pairing up with the 'wrong' morph themselves, no appreciable difference in fitness has been found (Cooke *et al.* 1995).

Another well-known example is the three species of *Stercorarius* skua that breed in the Northern Hemisphere and show plumage polymorphism. Of the three, the Arctic Skua (*Stercorarius parasiticus*) is perhaps the most studied. O'Donald (1983) in his study of a colony in Shetland, argued that sexual selection occurred with respect to morph because females showed a preference for pairing with melanic morphs. However, natural selection appeared to be acting on light morph males due to their earlier age at first breeding. Further work on other skua colonies has failed to find similar patterns of mate choice and life history (Phillips & Furness 1998).

Finding the genetic basis for colour polymorphism has been almost entirely lacking, but one species where the basis is known is the White-throated sparrow *Zonotrichia albicollis*. It occurs in two morphs, having either a white or tan eye stripe. Birds with a white stripe have a dominant pericentric inversion on chromosome 2 (Thornycroft 1975). Behavioural differences between morphs have been shown and include differing levels of extra-pair mating and differing levels of aggression (Tuttle 2003). Ninety-five percent of pairings are mixed-morph due to strong negative assortative mating. The population comprises of about half heterozygotes, which are white and half homozygous recessives. This population structure is the predicted equilibrium if one homozygote is fitter than the other (Falk and Li 1969) and the double inversion 'white-white' may be nearly lethal, as they are extremely rare.

In the Ruff, *Philomachus pugnax*, there is a genetic polymorphism that is associated with alternative mating behaviour at leks (Lank et al. 1995). Males of the two alternative tactics also exhibit differences in colouring of the elaborate breeding plumage. By rearing ruffs in captivity, Lank et al. (1995) showed that the morph development was consistent with a single-locus, two-allele autosomal genetic polymorphism.

Considering how these examples of avian colour polymorphisms fit into the four categories (apostatic selection, disruptive selection, non-random mating and no selection) listed by Galeotti et al. (2003), reveals that relatively little is known about the actual selection pressures leading to colour polymorphism. The lesser snow goose case possibly comes under the 'no selection' theory, with the ruff and white-throated sparrow being examples of non-random mating. The arctic skua is possibly a case of apostatic selection.

Krüger & Lindström (2001) have previously shown that the different morphs of buzzard have markedly different lifetime reproductive success, with intermediate individuals being almost twice as successful as the other two morphs. These less fit morphs appear to be maintained by Mendelian segregation, with the mode of inheritance appearing to be at a single-locus with two alleles, intermediates being the heterozygous form. Thus, this apparent heterozygote advantage in buzzards is most like the scenario that we see in the human example of sickle cell anaemia (Allison 1955, 1964).

Krüger et al. (2001) suggested that buzzards mated maladaptively with respect to morph. Light and dark individuals should preferentially pair up to produce all intermediate offspring. However, they showed that individuals preferentially paired up with birds of the same phenotype as their mothers, thereby meaning that light birds and dark birds would never pair up even though these pairings would produce only intermediate, maximally fit offspring. Young birds imprinting on the mother's phenotype most likely maintained this maladaptive mating.

Study Species

The Common Buzzard (*Buteo buteo*) is a medium-sized raptor (measurements: length 50-57cm, wingspan 113-128cm and weight 525-1364g (Cramp & Simmons 1980;del Hoyo *et al.* 1994)) found throughout the Palearctic region, from the Canary Islands through to Japan (see Fig. 1.1). Common Buzzards occur from sea level up to an altitude of approximately 1000 metres. They utilise a wide range of habitats, the only requirement being small patches of trees for roosting and breeding, although they have been recorded as nesting on the ground. Northern populations of buzzards are either fully or partially migratory. Wintering areas include sub-Saharan Africa, South East Asia and India. The degree of reversed sexual dimorphism is much less than in some species in the Family Accipitridae. Females are only 5-10% heavier and larger than males (Cramp & Simmons 1980).

The nominate race *B. b. buteo* which occurs in western Europe has the most variable plumage of any of the European Accipitridae (Glutz von Blotzheim *et al.* 1971;Cramp & Simmons 1980;del Hoyo *et al.* 1994). Three colour morphs have

been described and are readily identifiable in the field. These morphs are termed intermediate (Fig. 1.2), dark (Fig. 1.3) and light (Fig. 1.4).

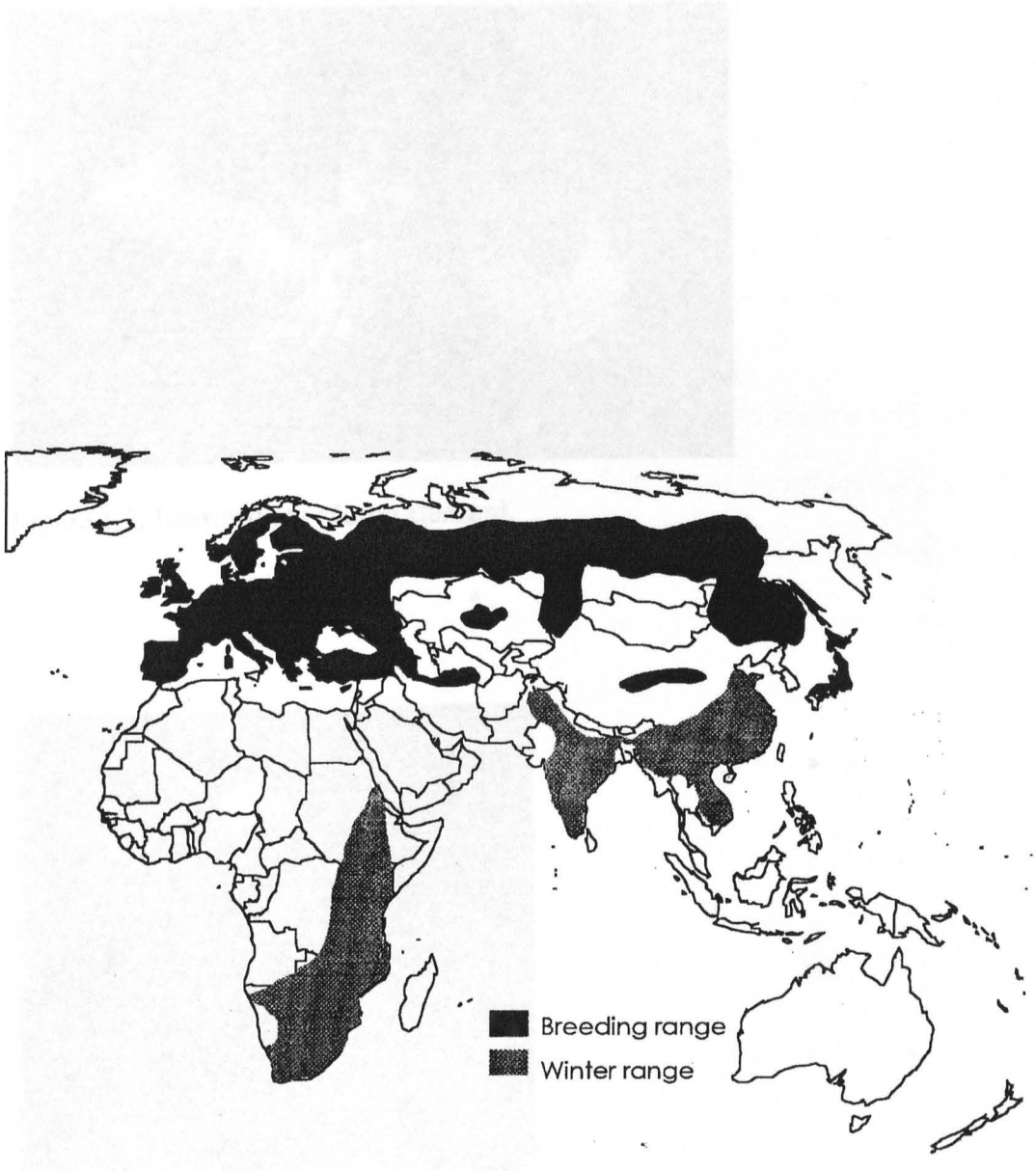


Figure 1.1: The distribution of the Common Buzzard (*Buteo buteo*).

Figure 1.2. Intermediate Morph Buzzard.

Figure 1.3. Dark Morph Buzzard

Figure 1.4. Light Morph Buzzard

Buzzards are usually monogamous and established pair bonds can last a lifetime (Cramp and Simmons 1980). Pairs occupy territories in late winter and laying occurs between late March and early May. Mean clutch size is roughly 2.6 (1-6) eggs (Cramp and Simmons 1980) and incubation lasts for 33-38 days. Females do most of the incubation while the male provisions with food. Chicks fledge 33-38 days after hatching (Cramp and Simmons 1980) but are fed by the parents for up to several weeks.

Study Site

Data on Common Buzzards has been collected since 1989 in a 300 km^2 area in Eastern Westphalia, Germany (see Fig. 1.5). This area lies between the towns of Osnabrück and Bielefeld. The area consists of three main habitats. In the north the area is heavily cultivated with a mosaic of agricultural fields, forest patches and meadows. Running diagonally through the middle is the Teutoburger Wald, which is a low-mountain forested area. In the south, there is another cultivated area, interspersed with forest patches that mainly consist of Scots Pine due to a more sandy soil type.

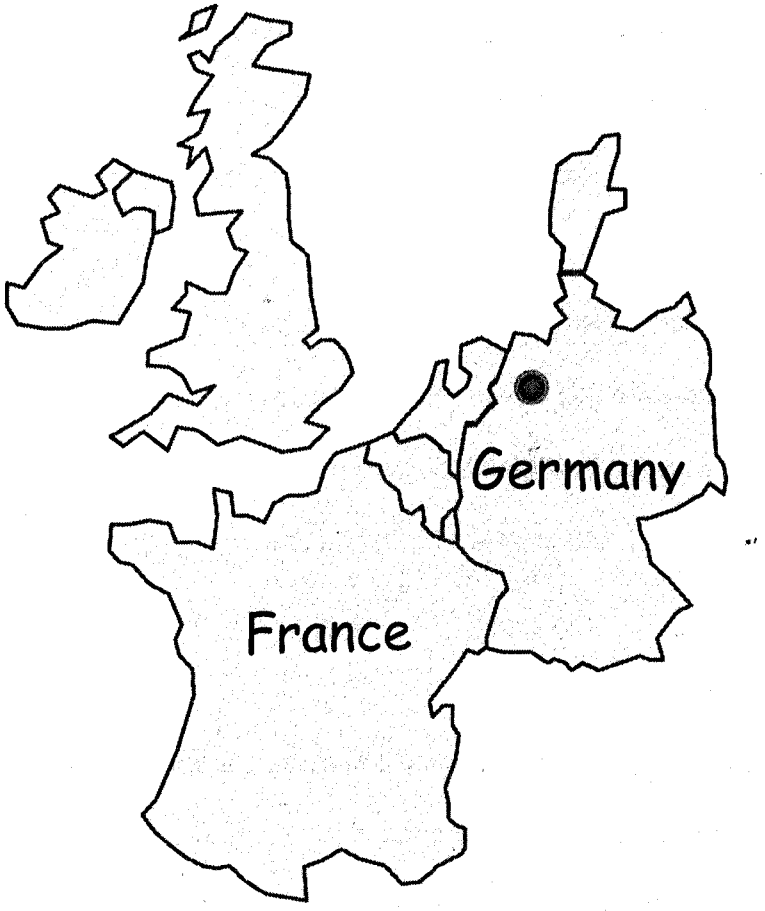


Fig. 1.5. Map showing the location of the study area

Questions addressed in this Thesis

This thesis investigates the evolution and life history consequences of colour polymorphism in the Common Buzzard. This study builds on the earlier work by Krüger et al. (2001) and extends it in various ways to further investigate the puzzle of the large fitness difference between the different morphs in the Common Buzzard.

To put this phenomenon into a broader evolutionary context, **Chapter 2** is a comparative analysis of raptors and owls and investigates whether traits associated with colour polymorphism are consistent with the avoidance-image hypothesis (Paulson 1973; Rohwer & Paulson 1987). This hypothesis suggests that a mutant new predator morph will invade a population because the prey does not recognise the new morph as readily as a predator compared with a common morph.

Once the polymorphism has arisen a mechanism is required to maintain it in a population. Assortative mating is one such mechanism. Buzzards have been shown to assortatively mate with respect to colour morph. **Chapter 3** consists of a modelling approach to assess whether buzzards do indeed mate maladaptively as has been previously proposed (Krüger et al. 2001).

Structured populations without a panmictic mating system may be more likely to suffer from differing levels of inbreeding. Inbreeding and its effects have been shown to have strong repercussions for lifetime reproductive success (Keller & Waller

2002). **Chapter 4** investigates whether differences in morph reproductive success are attributable to differing levels of inbreeding between the morphs.

Sometimes different morphs have different behaviours (Tuttle 2003). **Chapter 5** investigates behavioural differences between morphs and their potential effect on life history. Another behavioural trait is the timing of breeding and its association with variation in reproductive output. In **Chapter 6**, I investigate whether the timing of breeding can account for differences in reproductive output between morphs.

Chapter 7 consists of a general discussion of the work embodied in this thesis.

Chapter 2

THE EVOLUTION OF PLUMAGE POLYMORPHISM IN BIRDS OF PREY AND OWLS: THE APOSTATIC SELECTION HYPOTHESIS REVISITED

This chapter forms the basis of a paper published as Fowlie, M.K. & Krüger, O. 2003. The evolution of plumage polymorphism in birds of prey and owls: the apostatic selection hypothesis revisited. Journal of Evolutionary Biology **16**, 577-583.

ABSTRACT

Coevolution between phenotypic variation and other traits is of paramount importance for our understanding of the origin and maintenance of polymorphism in natural populations. We tested whether the evolution of plumage polymorphism in raptors and owls was supported by the apostatic selection hypothesis using ecological and life history variables in birds of prey and owls and performing both cross taxa as well as independent contrast analyses. For both bird groups, we did not find any support for the apostatic selection hypothesis: plumage polymorphism was not more common in taxa hunting avian or mammalian prey, nor in migratory species. In contrast, we found that polymorphism was related to variables such as population size and range size, as well as breeding altitude and breeding latitude. These results imply that the most likely evolutionary correlate of polymorphism in both bird groups is population size. This means that different plumage morphs might simply arise in larger populations most likely due to a higher probability of mutations.

INTRODUCTION

Phenotypic polymorphism independent of sex and age is found in a variety of taxa: fish (Franck *et al.*, 2001), reptiles (Losey *et al.*, 1997), birds (Huxley, 1955; Theron *et al.*, 2001) and mammals (Ritland *et al.*, 2001). Phenotypic polymorphism can be restricted to parts of the body (horn type: Gulland *et al.*, 1993, throat colour: Sinervo & Lively, 1996) or mean that entirely different skin or plumage phenotypes coexist in a population (O'Donald, 1983; Ritland *et al.*, 2001). The evolution and persistence of phenotypic polymorphism has challenged evolutionary ecologists for decades (Darwin, 1859; Mayr, 1963; Pemberton *et al.*, 1991; Lank *et al.*, 1995; Seehausen *et al.*, 1999). The challenge lies in explaining why directional selection on a trait as important as skin or plumage colour has failed to eliminate variation or, alternatively, why different morphs may be actually selected for.

Plumage polymorphism is especially common among certain bird taxa (Le Corre, 1999), such as birds of prey (Falconiformes), owls (Strigiformes) and skuas (Stercorariidae). Since these three groups are predatory, the widespread occurrence of polymorphism in these taxa has prompted evolutionary ecologists to propose hypotheses concerned mainly with foraging. The most widely accepted hypothesis is termed apostatic selection hypothesis or the avoidance-image hypothesis (Paulson, 1973; Rohwer, 1983; Rohwer & Paulson, 1987). The logic behind this hypothesis is appealing: a mutant new predator morph will invade a population because prey does not recognise the new morph as readily as a predator compared to a common morph. This selective advantage will lead to the new morph becoming more common until a frequency-dependent equilibrium is reached and the population is dimorphic

(Rohwer & Paulson, 1987). Support for this hypothesis would be if polymorphism was positively correlated with prey size (from insects over reptiles to birds and mammals) or hunting method (from preying on defenceless prey with low escape potential to prey with both high defence and escape potential).

While there has been some empirical support for the apostatic selection hypothesis (Paulson, 1973; Rohwer & Paulson, 1987), it has never been tested on a large scale or using modern comparative analysis techniques. However, it is clear now that a comparative analysis should address the problem of phylogenetic inertia, which biases any cross-taxa comparison (Harvey & Pagel, 1991). The fact that no thorough test of the avoidance-image hypothesis has been done so far is surprising, because the theory makes two clear predictions, which can easily be tested. First, polymorphism should be higher in species hunting birds or mammals (prey that has good vision as well as learning capabilities). Second, polymorphism should be higher in migratory species than in resident species, because migrants, being absent from a habitat for part of the year, invade a monomorphic population more easily.

As an alternative hypothesis, we tested whether polymorphism is merely a consequence of large population sizes, i.e. a presumably larger gene pool. Support for this hypothesis would be a positive correlation between polymorphism and measures of the gene pool such as population size and range size.

Our aim in this paper is hence straightforward: we test whether the two predictions of the apostatic selection hypothesis hold and, more generally, what the ecological or life history correlates of plumage polymorphism in birds of prey and owls are.

MATERIALS AND METHODS

We collected data on the 237 species in the family Accipitridae (true hawks) and on 58 species of owls (order Strigiformes) from the literature (mainly Thiollay, 1994; del Hoyo *et al.*, 1996; König *et al.*, 1999; Krüger, 2000; Ferguson-Lees & Christie, 2001). The dependent variable polymorphism was measured as the number of plumage morphs described for each species in Ferguson-Lees & Christie (2001) and König *et al.*, (1999). For those few species were an almost continuous variation in plumage has been reported, we entered the maximum number of morphs described in any species plus one (four for both birds of prey and owls). In addition, we included 24 predictor variables for birds of prey and 21 for owls (Table 2.1). A global world population estimate for each bird of prey species was obtained from Ferguson-Lees & Christie, 2001) and although such estimates get increasingly crude with increasing abundance, most birds of prey are now rare enough to make usefully accurate estimates. Egg volumes were estimated from egg length and breadth measurements in Schönwetter (1967-1992) and the approximation for egg volume provided by Hoyt (1979). Prey size categories were used to reflect prey size and in most cases there is about an order of magnitude in weight between prey size categories. The variable hunting method was included in order to reflect the energetic cost of hunting and the aerial skill level needed. The habitat preference variable was ranked from closed canopy habitat to increasingly more open and less productive habitat, hence there is some overlap with the habitat productivity variable. Global breeding range size was calculated for each species from information in Ferguson-Lees & Christie (2001) for

birds of prey and by overlaying the distribution maps of owls with world country maps and calculating the breeding range size from this comparison.

We performed both cross taxa analysis treating each taxon as an independent data point as well as calculating phylogenetically independent contrasts, using the method of Felsenstein (1985), as implemented in CAIC (Purvis & Rambaut, 1995). We included a cross taxa analysis because although formerly believed to yield erroneous conclusions (Harvey & Pagel, 1991), there is recent evidence that cross taxa analyses can be as statistically valid and as biologically informative as independent contrasts (Price, 1997; Harvey & Rambaut, 2000). The comparative analyses for birds of prey were based on the osteological phylogeny of genera by Holdaway (1994) and a molecular phylogeny of species by Wink & Sauer-Gürth (2000). For owls, we used a molecular phylogeny of species provided by Wink & Heidrich (1999). All three phylogenies provide estimates of branch lengths that were used in the analyses.

We developed multivariate stepwise regression models for both groups in SPSS. The models for independent contrasts did not include an intercept, as recommended by Harvey & Pagel (1991). To address the problem of multicollinearity, we looked at tolerance levels and only included variables above 0.1 tolerance, as recommended by Hair *et al.*, (1995). Models were only considered valid if residuals were distributed normally (James & McCulloch, 1990).

Table 2.1 Explanatory variables included in the analyses and their description.

Variable	Description	Birds of prey	Owls
Body weight	Log body weight [g]	+	+
Body size	Body size from tip of bill to tip of tail [cm]	+	+
Wingspan	Wingspan [cm]	+	
Wing length	Wing length [cm]	+	+
Tail length	Tail length [cm]	+	
Sex size dimorphism	Male wing length over female wing length cubed	+	+
Sex plum. dimorphism	Scored from 0-4 (Krüger & Davies 2002)	+	+
Population density	Number of breeding pairs/100 km^2	+	+
Population size	Log world population estimate in breeding pairs	+	
Breeding system	-1=polygyny, 0=monogamy, 1=polyandry	+	+
Egg volume	Estimated egg volume [ml]	+	+
Clutch size	Mean clutch size	+	+
Incubation time	Mean incubation time [days]	+	+
Fledging time	Mean fledging time [days]	+	+
Reproduction rate	Mean number of chicks fledged/pair and year	+	+
Prey	1=fruits, 2=insects, 3=snails, 4=frogs, 5=lizards, 6=snakes, 7=fish, 8=rodents, 9=birds, 10=small carrion, 11=mammals, 12=large carrion	+	+
Prey specialisation	Scored from 1 (opportunist) to 4 (extreme specialist) (Krüger 2000)	+	+
Hunting method	Scored from 1 (only searching) to 4 (attacks on agile prey with defence potential (Krüger 2000)	+	+
Habitat preference	1=tropical forest, 2=subtropical & temperate forest, 3=woodland, 4=freshwater habitats, 5=coastline, 6=marsh, 7=savannah, 8=grassland, 9=mountain, 10=semi-desert	+	+
Habitat productivity	Productivity in g carbon m^{-2} per year (Reichle 1970)	+	+
Breeding altitude	Median breeding altitude above sea-level [m]	+	+
Breeding latitude	Median breeding latitude [°]	+	+
Migration pattern	Scored from 0-4 (Krüger & Davies 2002)	+	+
Range size	Log global breeding range size [km^2]	+	+

RESULTS

Birds of prey

Out of the 237 species, 72 or 30% showed some degree of polymorphism. Across species, the number of morphs described was significantly correlated with eight out of the 24 explanatory variables (Table 2.2). The degree of polymorphism increased with sexual plumage dimorphism, world population size, reproduction rate, migration pattern and range size while it decreased with incubation and fledging period as well as habitat productivity. This first analysis supported the second prediction of the avoidance-image hypothesis that migratory species should be more polymorphic. Across genera, the correlation pattern changes considerably. Seven out of the 24 explanatory variables were significantly correlated with the degree of polymorphism (Table 2.2). across species, namely that polymorphism increased with sexual plumage dimorphism and reproduction rate. Polymorphism decreased with body weight, body size, wingspan and wing length. In addition, polymorphism decreased with prey size, contrary to the prediction made by the avoidance-image hypothesis. These results were not changed when the carrion-feeders were excluded from the analysis (feeding on large dead prey renders predator recognition impossible and might bias results).

Table 2.2: Cross taxa correlations between polymorphism and explanatory variables for birds of prey.

	Cross species		Cross genera	
Variable	n	r	n	r
Body weight	197	-0.078	62	-0.263*
Body size	237	-0.085	65	-0.253*
Wingspan	232	-0.070	65	-0.277*
Wing length	232	-0.047	65	-0.271*
Tail length	232	-0.049	65	-0.133
Sexual size dimorphism	232	-0.038	65	0.014
Sexual plumage dimorphism	237	0.186**	65	0.420***
Population density	145	0.036	59	-0.070
Population size	237	0.263***	65	0.156
Breeding system	237	-0.027	65	-0.042
Egg volume	163	-0.083	57	-0.194
Clutch size	190	0.084	59	0.116
Incubation time	148	-0.254**	52	-0.187
Fledging time	141	-0.177*	50	-0.195
Reproduction rate	115	0.193*	48	0.301*
Prey size	232	-0.102	64	-0.318*
Prey specialisation	232	-0.048	64	0.025
Hunting method	232	0.004	64	0.006
Habitat preference	237	0.011	65	-0.211
Habitat productivity	237	-0.129*	65	0.194
Breeding altitude	237	0.071	65	-0.148
Breeding latitude	237	0.122	65	0.034
Migration pattern	237	0.198**	65	-0.026
Range size	237	0.216***	65	0.121

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ Only two correlations were also found

The independent contrast analysis at the species level identified three explanatory variables that co-evolved with the degree of polymorphism (Table 2.3). The multiple regression model was highly significant ($F_{3,50} = 7.555$, $p < 0.0001$) and explained 31.2% of the variation in polymorphism degree. Polymorphism increased with sexual plumage dimorphism and world population size while it decreased with breeding altitude.

Shifting to the genera level, the independent contrast analysis identified two explanatory variables, which co-evolved with polymorphism (Table 2.3). The corresponding model was highly significant ($F_{2,38} = 7.299$, $p = 0.002$) and explained 38.9% of the variation in polymorphism degree. Polymorphism increased with both world population size and breeding latitude. World population size was also a predictor in the species` contrast model. In addition to these two variables which entered the model, polymorphism was also significantly correlated with breeding system, reproduction rate, incubation and fledging period and range size. Polymorphism increased from polygynous over monogamous to polyandrous breeding systems, increased with reproduction rate and range size while it decreased with incubation and fledging period.

Table 2.3: Multiple regression models for birds of prey species (top) and genera (bottom). The s.e. are 0.248 and 0.049 respectively and residuals are normally distributed.

Variable	β	s.e	t	p	R^2	Collinearity
Species contrasts (n = 53)						
Sex plumage dimorphism	0.334	0.115	2.912	0.005	0.179	0.960
Breeding altitude	-0.0003	0.0001	2.199	0.033	0.257	0.963
Population size	0.169	0.084	2.012	0.048	0.312	0.978
Genera contrasts (n = 40)						
Population size	0.228	0.064	3.563	0.001	0.325	0.986
Breeding latitude	0.007	0.003	2.144	0.043	0.389	0.986

Owls

Out of the 58 species included, 29 or 50% showed some degree of polymorphism. Across owl species, the degree of polymorphism was related to eight out of the 21 explanatory variables (Table 2.4). Polymorphism increased with migration pattern while it decreased with body weight, body size, wing length, incubation and fledging period, prey size and hunting method. Again, correlations with prey size and also hunting method were negative, contrary to the prediction made by the avoidance-image hypothesis. However, like in the birds of prey cross-species analysis, more polymorphic species were also more migratory.

The independent contrast analysis produced a multiple regression model, which included three explanatory variables, namely range size, wing length and habitat preference (Table 2.5). The model was highly significant ($F_{3,54} = 12.696$, $p < 0.0001$) and explained 41.4% of the variation in polymorphism. Polymorphism increased with range size and more open, less productive habitat while it decreased with wing length. Range size was also a significant correlate in the raptor genera contrast analysis.

Table 2.4: Cross species correlations between polymorphism and explanatory variables for owls.

Variable	n	r
Body weight	58	-0.291*
Body size	58	-0.306*
Wing length	58	-0.279*
Sexual size dimorphism	58	0.124
Sexual plumage dimorphism	58	0.015
Breeding system	48	0.164
Population density	49	0.178
Reproduction rate	47	0.059
Egg volume	48	-0.226
Clutch size	58	-0.117
Incubation time	58	-0.423***
Fledging time	58	-0.295*
Prey size	58	-0.331*
Prey specialisation	58	0.051
Hunting method	58	-0.404**
Habitat preference	58	0.001
Habitat productivity	58	0.079
Breeding altitude	58	-0.115
Breeding latitude	58	-0.110
Migration pattern	58	0.264*
Range size	58	0.194

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Table 2.5: Multiple regression model for owl species contrasts (n = 57). The s.e. is 0.279 and residuals are normally distributed.

Variable	β	s.e.	t	p	R^2	Collinearity
Range size	0.694	0.140	4.968	0.001	0.173	0.946
Wing length	-0.008	0.002	3.758	0.001	0.345	0.782
Habitat preference	0.157	0.063	2.506	0.015	0.414	0.787

DISCUSSION

Our comparative analyses do not support the apostatic selection hypothesis (Paulson, 1973; Rohwer & Paulson, 1987). Neither in birds of prey nor in owls was any variable related to foraging a significant predictor of polymorphism levels. For this hypothesis to be supported, we would have predicted a positive correlation between prey size and/or hunting method and polymorphism level. This is due to the fact that birds and mammals constitute not only the largest prey categories but were also assumed to be those prey types which could learn to recognise a predator and hence would select for polymorphism in the predator (Paulson, 1973; Rohwer, 1983). One could argue that earlier studies (Paulson, 1973; Rohwer, 1983), proposing the apostatic selection hypothesis relied on cross taxa analysis whereas we used independent contrasts. However, even in the cross taxa analyses we have done here, prey size was negatively related to polymorphism levels: this means that species hunting insects, amphibians and reptiles had higher polymorphism levels compared to those hunting birds and larger mammals. This already indicates that rather than prey, population size might be important because species feeding on smaller prey can achieve higher local and global population sizes than species feeding on large prey (Newton, 1979, see also below). Species feeding on a variety of prey (generalists) should be able to achieve higher population sizes than feeding specialists, but we did not find any significant negative correlation between prey specialisation level and plumage polymorphism. The other prediction made by the apostatic selection hypothesis, that migratory species should be more polymorphic than resident species (Rohwer & Paulson, 1987) is supported by the cross taxa analysis of birds of prey

and owls (we found a positive correlation between migration pattern and polymorphism level in both groups), but for both taxa there was no correlation using independent contrasts.

Our results strongly indicate that polymorphism is related to population size in birds of prey, and also in owls. While we did not have a world population estimate for owls, one strong predictor of polymorphism levels in owls was range size, which is known to be tightly correlated with population size in many raptors (Ferguson-Lees & Christie, 2001). Other predictor variables also indicate the paramount importance of population size. In birds of prey, polymorphism decreased with breeding altitude and increased with breeding latitude. Clearly, species breeding at lower altitude can achieve a larger range size and population size and it is well known that species` ranges increase from the equator to the poles (Rapoport`s rule, Rapoport, 1982). Even habitat preference, an important predictor variable for owls, might be related to population size. We found that owls preferring more open, less productive habitats had a higher polymorphism level than those living in closed forests. This might reflect again larger ranges and populations in temperate species compared to tropical rainforest species.

One might argue that if population size is such an important variable linked to polymorphism, why is polymorphism not much more common among passerine species, many of which attain far larger population sizes than any raptor? This ignores that predation is a powerful selection pressure. Predation however, does not greatly affect birds of prey and owls since even in the smaller species, predation pressures are weak, certainly compared to other bird groups. The notion that

polymorphism is more common in birds of prey, owls and skuas might simply also reflect that these three groups do not face strong selection from predation to reduce phenotypic variability. While we are the first to admit that inferences from comparative analyses should be treated with caution, because these types of analyses cannot distinguish between cause and effect (see, however Krüger & Davies, 2002) and the problem of differences in data quality between species, we believe that our main conclusion about the importance of the apostatic selection hypothesis is not greatly affected by these limitations.

Finally, sexual plumage dimorphism was a significant positive predictor for polymorphism levels in birds of prey. This indicates that sexual selection might be a precursor for phenotypic diversity, because plumage differences between the sexes are often assumed to be the result of sexual selection (Andersson, 1994; Barraclough *et al.*, 1995). If errors occur during recombination, the sex-linked genes responsible for dimorphism might become integrated into other chromosomes and polymorphism might arise independent of sex (Thorne *et al.*, 1997).

In conclusion, our results show that polymorphism simply arises more readily in large populations, but this says nothing about the mechanisms to retain it within a population. It is unlikely that such an obvious phenotypic trait is selectively neutral, hence there might be species-specific mechanisms to maintain phenotypic diversity. They can vary from mating preferences (O`Donald, 1983; Phillips & Furness, 1998), mating strategies (Lank *et al.*, 1995; Sinervo & Lively, 1996), balancing genetic inheritance (Krüger *et al.*, 2001), to a combination of different selection pressures (Forsman & Shine, 1995; Losey *et al.*, 1997; Franck *et al.*, 2001). The appealing and

simple hypothesis that prey learns to recognise a predator which in turn selects for phenotypic diversity is unlikely to be generally true.

Chapter 3

ARE BUZZARDS MATING MALADAPTIVELY? MODELLING THE INTERACTION BETWEEN MATING STRATEGIES AND POPULATION DEMOGRAPHY

This chapter forms the basis of a paper in review in Evolution as:

Fowlie, M.K., Lindström, J. & Krüger, O. Are buzzards mating maladaptively? Modelling the interaction between mating strategies and population demography

ABSTRACT

The Common Buzzard shows three plumage colour polymorphisms, each one having significant differences in lifetime reproductive success. It has previously been shown to mate maladaptively with respect to which colour morph it chooses as a mate. Here we compare the observed mating pattern of mating with one's mother's phenotype by using a demographically structured population model as a basis for an evolutionary invasion analysis. Two scenarios were modelled, (i) preferred mates are always available, and (ii) mate availability depends on the prevailing demography in the population. The mating strategies competing with the observed one are random, mating with one's own phenotype, mating with an individual dissimilar to one's mother's phenotype and mating to maximise fitness. We show that buzzards do indeed appear to mate maladaptively but only if we assume that the genes for mate choice are aware of which phenotype they reside in. If this is not the case due to potential genetic constraints, buzzards do indeed mate adaptively although the observed strategy appears to be 'the best of a bad job.' We also show that mate choice has implications for the population demography, which in turn affects an individual's ability to secure preferred mates.

INTRODUCTION

Choosing a mate is one of the most important decisions in an individual's lifetime. Theoretical and empirical studies show that the choice can have strong repercussions on reproductive fitness (Kokko 1998; West & Packer 2002), in both the short and the long term. Optimality is predicted in mate choice but real examples remain rare (Andersson 1994). The potential advantages of discriminating between mates are many and include species recognition, improved offspring viability, gaining a mate with better parental ability, or mate that better complements its partner. The majority of genetic models for mate choice concentrate on the heritable aspects of mating success and viability, however it is possible for non-genetic benefits to favour mate choice too (Kirkpatrick & Ryan 1991; Thornhill & Alcock 1983). Demonstrating genetic benefits from mate choice has not been easy and those that are found are often of minor consequence or unclear (Doty & Welch 2001; Brooks 2000; Jones *et al.* 1998). This, coupled with the fact that females may allocate differential resources to offspring (Cunningham & Russell 2000), can blur some supposed genetic benefit results. However, by examining the reverse situation i.e. the effects of inbreeding, (Keller & Waller 2002), where deleterious effects are known (Slate *et al.* 2000) we can clearly see the potential of genetic benefits from mate choice. Indeed, mate choice can take the form of avoiding certain individuals, or individuals can mate with individuals dissimilar to themselves to maximise the heterozygosity of their offspring (Brown 1997).

Life-history theory would predict that individuals should maximise the reproductive value of offspring depending on their state or the environment (Brommer *et al.*

2000). Sex allocation theory does this for the manipulation of offspring sex (Hardy 2002), but examples of the parental manipulation of other offspring states where fitness differences occur are rare.

In the common buzzard (*Buteo buteo*), the benefits of mate choice are unusually clear. European buzzards are highly variable in colour and three main morphs have been distinguished (Glutz von Blotzheim *et al.* 1971;Melde 1983). The frequencies of morphs tend to vary across Europe; nevertheless some generalisations can be made. The dark morph is always the rarest morph ranging from 0.1% (Dittrich 1985) to 17% (Zang *et al.* 1989). The light morph is slightly more common than the dark morph ranging from 2.3% (Dittrich 1985) to 21% (Zang *et al.* 1989). The intermediate morph is always the most common ranging from 60% (Zang *et al.* 1989) to 98% (Dittrich 1985). Of the three morphs, those of intermediate colour enjoy much higher lifetime reproductive fitness than the two extreme morphs (Krüger & Lindström 2001a), being both more fecund and longer lived.

Krüger *et al.* (2001) recently showed that plumage color seemed to be controlled for at a single locus with light birds having two pale form alleles and dark birds two dark, with intermediates being heterozygous. It was also proposed that the common buzzard appears to mate maladaptively due to the fact that light and dark birds do not preferentially pair up to produce all intermediate offspring, thereby seemingly maximising their lifetime reproductive fitness. Their mate choice modelling showed that only the strategy of pairing with one's mother's phenotype produced a good fit with the data. It was argued that this strategy was being maintained by the imprinting of the offspring on the mother, this species recognition cue then having secondary

effects on mate choice later in life. Here we compared the observed strategy to four others by using a more rigorous evolutionary invasion analysis approach (pairing at random, pairing to maximise fitness, pairing with own phenotype and pairing with a phenotype dissimilar to one's mother) to see if individuals do indeed mate maladaptively, how different the different strategies would actually be, and how the resulting demographies would differ.

MATERIALS AND METHODS

The overall fitness of the mother's phenotype strategy was compared against four others: random pairing, pairing to maximise fitness, pairing with one's own phenotype and pairing with an individual dissimilar to one's mother. Random pairing is a self-explanatory strategy, in which a female is assumed to accept any available male. In the maximising fitness strategy both light and dark morphs preferentially pair up with each other to produce the all intermediate offspring. Intermediate birds preferentially pair up with light birds to produce 50% intermediate and 50% light offspring. Finally, it is also possible that individuals follow the kind of assortative mating where they preferentially pair up with a mate of a similar phenotype or one that is dissimilar from their mother.

As the buzzard colour morphs differ not only in their fecundity but also in their survival (Krüger & Lindström 2001a), to be able to compare the strategies, the number of offspring produced by a given morph was weighted by the reproductive values of the different offspring morphs (Caswell 2001). This weighted sum gives us

a fitness measure that takes into account the effectiveness of a given offspring to convey parental genes into future generations. The reproductive values of offspring produced into different morphs were calculated by finding the left eigenvector, **v**, of the transition matrix (Caswell 2001) separating between juvenile individuals and breeding adults of each morph:

$$\mathbf{A} = \begin{bmatrix} 0 & 0 & 0 & F_{LL} & F_{IL} & 0 \\ 0 & 0 & 0 & F_{LI} & F_{II} & F_{DI} \\ 0 & 0 & 0 & 0 & F_{ID} & F_{DD} \\ P_{YL} & 0 & 0 & P_{AL} & 0 & 0 \\ 0 & P_{YI} & 0 & 0 & P_{AI} & 0 \\ 0 & 0 & P_{YD} & 0 & 0 & P_{AD} \end{bmatrix}. \quad (1)$$

Here, each fecundity entry F_{ij}, is formed by calculating:

$$\alpha_{iL}\beta_i p_i f_L + \alpha_{jI}\beta_i p_i f_I + \alpha_{jD}\beta_i p_i f_D, \quad (2)$$

where α_{ij} is the probability that a male of morph i pairs up with a female of morph j (this probability is thus dependent on the mate choice strategy and the frequency of the desired partners within the population), βi denotes the probability that an offspring of morph i is born for this pair α_{ij} (Krüger *et al.* 2001), p_i is the survival probability for a new-born individual to enter into the yearling age-class, and f_i gives the morph-specific female fertility. P_{Yi} and P_{Ai} values denote morph-specific annual survival probabilities for yearlings and adults, respectively. As we do not have direct estimates for the survival of offspring from fledging until their first breeding attempt, we used the value 0.536 (Cramp & Simmons 1980) as was done in an earlier analysis based on the same study (Krüger & Lindström 2001a). Morph specific adult survival values (P_{Ai}) were 0.482 for light, 0.722 for intermediate and 0.466 for dark birds.

These values are calculated from observed individuals within the study population (Krüger & Lindström 2001b). For morph-specific fertility, f_i, we used the weighted average (age-specific fertilities weighted by the sample size) over the breeding career of light, intermediate and dark females: 0.93, 1.67, and 0.54, respectively. Assuming 50:50 sex ratios among offspring, we divided these fertility values by two as we are modelling only the demography of female part of the population. The fitness Λ_{ij} for a female of morph i under mating strategy j then becomes

$$\Lambda_{ij} = r_L N_L + r_I N_I + r_D N_D, \quad (3)$$

where N_i denotes the part of morph-specific fertility producing an offspring of a given morph, and r_i are the reproductive values of the corresponding morph's yearlings which are then used to weight the value of a given offspring.

However, the different reproductive values of offspring of different morphs is not the only determinant of a given mate choice strategy — also the population demography changes if different mate choice strategies are assumed and consequently, different frequencies of desirable partners are available (see Table 3.1 for illustration of this point). Therefore, two different scenarios were used. 'Free market', where individuals are always able to pair with their preferred choice under each of the four strategies, and 'Real Life', where the demography of the resident strategy influenced the availability of partner.

The overall fitness value for a given strategy under a given scenario was calculated as the sum of the three morph fitnesses under that strategy (see Appendix), as all morphs contribute to the strategy. Preference values for each possible pairing were assigned. These were based on the morph frequencies of a given population coupled with a weighted probability depending on the strategy and partially derived from the reproductive values.

The stable age structure of the population following a resident strategy was calculated as the right eigenvector, **w**, of the transition matrix (Eq. 1) (Caswell 2001). Invasion analysis of each strategy was then performed, assuming that the mutant strategy is initially so rare that its effect on the population demography can be ignored (Houston & McNamara 1999). This involved introducing a mutant of one strategy into the resident strategy and seeing that given the population demography whether the mutant strategy's expected fitness was greater than that of the resident strategy. As the strategy fitness was calculated using the reproductive values, an invasion thus means that a mutant strategy would increase in numbers in a population of individuals following the original, resident strategy (see, e.g., McNamara 1993, Houston & McNamara 1999). As the invasion criteria are based on the reproductive values derived from the transition matrix, the invasion analysis was deterministic and density-independent. This was for two reasons, firstly the observed population on which the model was based is relatively stable (population growth rate is close to 1) and therefore the assumptions of the matrix analysis are not violated (Krüger & Lindström, 2001). Secondly, as the population is so stable, a convincing analysis of possible density dependencies in life-history traits is impossible — especially as the

resolution of the analysis should probably be at the morph level given the huge differences between the morphs. It was assumed that once a strategy had invaded the population stable age structure would stabilize before another strategy invasion would happen. The actual numerical values of strategy fitness are not informative as they are not comparable between strategies due to the way in which the left eigenvectors, **v**, of the matrices were calculated (they are relative contributions to long-term population growth, and scaled so that a chosen stage is given a value of 1; Caswell 2001). However, an invasion hierarchy using all possible combinations of resident and mutant strategies can be built, indicating which strategy can invade which. It is also important to note that each successive invasion changes the population demography thereby altering the ratio of morphs, which in turn influences mate choice and the ability of new strategies to invade.

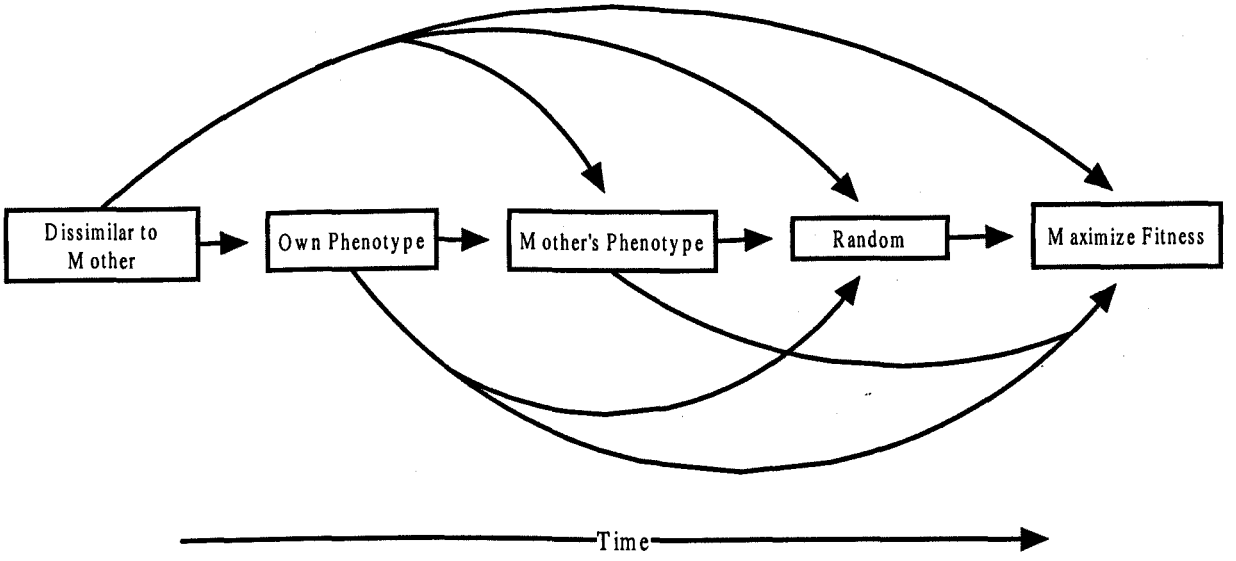
RESULTS

Under the 'free market' scenario (Fig. 3.1a) pairing with an individual dissimilar to one's mother is invaded by all the four other strategies. The next best strategy is pairing with one's own phenotype, invaded by three others. This is invaded by pairing with one's mother's phenotype which in turn is invaded by random pairing which is invaded by maximise fitness strategy.

Under the 'real world' scenario (Fig. 3.1b) pairing with an individual dissimilar to one's mother is invaded by the four other strategies. The next best strategy is random pairing invaded by three others. Of these three strategies invading random mating, mother's phenotype can only be invaded by maximise fitness strategy. Random

mating can also be directly invaded by maximise fitness strategy. If random mating is invaded by mate with your own phenotype strategy, this can be invaded by mother's phenotype strategy which in turn can be invaded by maximise fitness strategy, or it can be directly invaded by maximise fitness strategy. This gives a hierarchy of strategies with respect to fitness. Table 1 shows the changes in proportions of the different states depending on the strategy. For example the proportion of intermediate adults (*ai*) varies from 0.52 under pairing with own phenotype strategy to 0.72 under pairing with phenotype dissimilar to mother's.

a)



b)

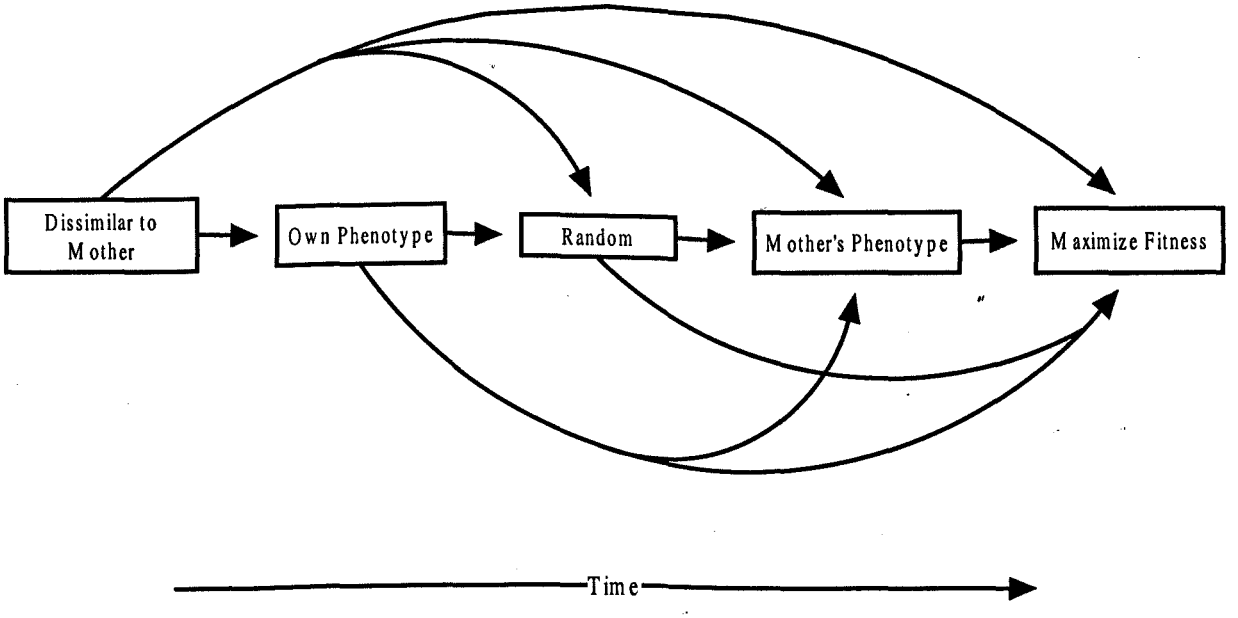


Fig. 3.1 Invasion hierarchies for the two different scenarios "free market" (A), and the "real world" (B). A strategy further away in time can always invade the strategy that was previously the resident one, and the arrows therefore show which strategy could mutate to which.

Table 3.1: Stable age structures (w), Reproductive values (v) and Population growth rates (λ) for each mate choice scenario.

	State	Random	Non mother	Own	Mother	Max
w	*jd*	0.0406	0.0267	0.0630	0.0545	0.0394
	jl	0.0485	0.0326	0.0667	0.0631	0.0483
	ji	0.0888	0.0776	0.1217	0.1175	0.0905
	ad	0.0832	0.0587	0.1084	0.0971	0.0803
	al	0.1036	0.0750	0.1187	0.1165	0.1027
	ai	0.6354	0.7294	0.5216	0.5514	0.6388
v	*jd*	1.0000	1.0000	1.0000	1.0000	1.0000
	jl	1.7940	1.7994	1.8310	1.9496	1.5663
	ji	10.8772	13.9144	99.1170	7.9120	8.9584
	ad	1.1254	1.0920	1.2189	1.1987	1.1274
	al	2.0189	1.9650	2.2318	2.3370	1.7658
	ai	12.2407	15.1945	120.8150	9.4840	10.0993
λ		0.8239	0.7995	0.8924	0.8776	0.8253

DISCUSSION

We have compared different mate choice strategies in the common buzzard by the means of invasion analysis based on an individual-based long-term data set and demographic modelling. As the common buzzard shows genetic, persistent colour polymorphism where individuals of intermediate pigment enjoy much higher lifetime reproductive success than either of the extreme morphs, it is of interest to see whether the individuals aim to maximise the number of maximally fit offspring produced (Krüger & Lindström 2001; Krüger et al. 2001). If parents can produce offspring into different states, they are expected to maximise the sum reproductive value of their offspring instead of their number (Houston & McNamara 1999). Appealing as this idea is, good examples are rare. As the expected fitness value of the different types of offspring in buzzards depend heavily on their state (morph), this situation serves us as an unusual opportunity to study strategy fitness based on reproductive values. Our analysis should therefore reveal the mate choice strategy that is realistically as close to an evolutionary optimum as can be shown, based on empirical data.

A parallel idea in evolutionary ecology is that of sex allocation theory (Hardy 2002). In this, individuals manipulate the sex ratio of their offspring depending on environmental conditions and the quality of mate to maximise the reproductive value of those offspring. Individuals can do this either pre- or post zygotically. As morph determination has a simple genetic basis and we have no information on differential allocation to chicks of different morphs, we believe that mate choice is the only means available to maximise the reproductive value of offspring.

We modelled the mate choice in two different scenarios, under "free market" where the desired type of mate is always available, and under the "real world" scenario where the number of available mates is limited by demography and competition by the other individuals.

In both of these scenarios, pairing with an individual dissimilar to your mother is the worst strategy for a buzzard to use. This is due to the fact that because intermediate morphs are the most prevalent, not only is an individual most likely to be an intermediate but so is its mother. So by pairing with an individual dissimilar to it's mother a bird is more likely to pair with an individual that is detrimental to its reproductive success, i.e., one of the extreme morphs.

Under a 'free market' scenario (Fig 3.1a) where each individual is always able to find its preferred choice, the observed mate choice strategy of preferentially pairing up with one's mother's phenotype (Krüger et al. 2001) appears to be profoundly maladaptive. Random mating will invade because this gives individuals a slightly higher chance of pairing the optimal partner, and mating to maximise fitness strategy also invades. However, this scenario is flawed because frequencies of the different colour morphs vary within the population (see Table 3.1) and so the chances of actually getting your preferred partner are much lower.

However, under the 'real world' scenario (Fig 3.1b) where an individual's choice is influenced by observed differences in morph frequencies governed by the population demography, the mother's phenotype strategy is only invaded by mating to maximise fitness strategy.

Thus, the mate choice strategy that the buzzards seem to follow in nature where they prefer their mother's phenotype (Krüger et al. 2001) appears at first glance to be maladaptive: individuals do not make the seemingly correct choice of passing as many genes to the next generation as possible. However, implicit in the maximising fitness strategy is the assumption that genes for mate choice are aware of the phenotype that they reside in. Light individuals should prefer dark ones, and vice versa, however the intermediate individuals should prefer light mates over dark ones to avoid producing the worst offspring combination. An individual must therefore assess its own colour before making a decision with regard to potential partners, and this strategy is not a simple rule of thumb. For the plastic nature of this strategy to evolve, a very close link between genes for mate choice and those for color polymorphism would have to exist. Due to crossovers during recombination and other copying errors it may not be possible to maintain such a tight linkage between the two sets of genes, and therefore the evolution and/or maintenance of this strategy in a population is perhaps impossible (Ayala 1976).

The mother's phenotype strategy is a general rule applicable to all individuals irrespective of morph and seemingly can be maintained by the imprinting of individuals on their mothers from an early stage in their development. Genetic constraints imposed on the buzzards mean that although individuals could improve their lifetime reproductive success by using the maximise your fitness strategy, they may be unable to do so. So within the imposed constraints individuals are in fact mating optimally and maximising the sum of their offspring reproductive value even though at first glance they appear not to do so.

APPENDIX

Symbols used in the equations:

L = Light Morph, D = Dark Morph, I = Intermediate Morph

V = Reproductive Value, F = Fecundity,

C_A, C_B, C_C = Preference choice, with A>B>C

R = Random Mating Strategy

X = Maximise Fitness Strategy

O = Pair with one's own phenotype strategy

S = Pair with mother's phenotype strategy

M_x = Probability that mother was morph x

W_x = Proportion of birds in population of morph x, derived from the stable age structure

Y_x = Proportion of young of morph x produced in a given scenario

To illustrate the calculations involved in determining the fitness of a morph under a certain mating strategy, we show how we calculated the fitness for Light individuals under Random mating. The rest of the situations modelled were solved in the same way. Light individuals can only produce light or intermediate offspring from pairing with any of the three available morphs. The fitness accrued from producing light offspring is calculated as $\left(V_L F_L \left[\left(C_A W_L Y_L\right) + \left(C_A W_I Y_L\right)\right]\right)$. Here the probability of encountering a given mate (W_x) is coupled with the proportion of light young produced in that mating (Y_L). In the random mating scenario the preference value of

a particular pairing (C_A), each value is equal irrespective of the choice. This weighted sum of light offspring produced when pairing either with light or intermediate partner is then multiplied by the fecundity of the light adult (F_L) and finally scaled by the reproductive value of the light offspring (V_L). The same steps are repeated for calculating the production of intermediate offspring by a light bird: $\left(V_I F_L \left[(C_A W_I Y_I) + (C_A W_D Y_I)\right]\right)$, and the two are summed up to give an overall fitness for light birds under a random mating strategy. Similar equations are generated for intermediate and dark birds and the combined total value for the three morphs give us a strategy fitness value.

Equations are given for strategy fitness for light and intermediate morphs only. Equations for the dark morph are essentially the same as for light. Strategy equations for pair with a dissimilar phenotype to mother are the inverse for those shown for Pair with mother's phenotype strategy.

The fitness for Light and Intermediate individuals under Random Mating Strategy can be shown as:

$$L_R = \left(V_I F_L \left[(C_A W_I Y_I) + (C_A W_D Y_I)\right]\right) + \left(V_L F_L \left[(C_A W_L Y_L) + (C_A W_I Y_L)\right]\right)$$

$$I_R = \left(V_I F_I \left[(C_A W_I Y_I) + (C_A W_L Y_I) + (C_A W_D Y_I)\right]\right) + \left(V_L F_I \left[(C_A W_I Y_L) + (C_A W_L Y_L)\right]\right) + \left(V_D F_I \left[(C_A W_I Y_D) + (C_A W_D Y_D)\right]\right)$$

The fitness for Light and Intermediate individuals under Maximise Fitness Strategy can be shown as:

$$L_X = \left(V_L F_L \left[(C_B W_I Y_L) + (C_C W_L Y_L)\right]\right) + \left(V_I F_L \left[(C_A W_D Y_I) + (C_B W_I Y_I)\right]\right)$$

$$I_X = \left(V_D F_I \left[(C_B W_I Y_D) + (C_C W_D Y_D)\right]\right) + \left(V_L F_I \left[(C_A W_L Y_L) + (C_B W_I Y_L)\right]\right) + \left(V_I F_I \left[(C_A W_L Y_I) + (C_B W_I Y_I) + (C_C W_D Y_I)\right]\right)$$

The fitness for Light and Intermediate individuals under Pair with Mother's Phenotype Strategy can be shown as:

$$L_S = \left(V_L F_L \begin{bmatrix} (C_A M_L W_L Y_L) + (C_B M_L W_I Y_L) \\ + (C_B M_I W_L Y_L) + (C_A M_I W_I Y_L) \end{bmatrix}\right) + \left(V_I F_L \begin{bmatrix} (C_B M_L W_I Y_I) + (C_B M_L W_D Y_I) \\ + (C_A M_I W_I Y_I) + (C_B M_I W_D Y_I) \end{bmatrix}\right)$$

$$I_S = \left(V_D F_I \begin{bmatrix} (C_A M_D W_D Y_D) + (C_B M_D W_I Y_D) \\ + (C_A M_I W_I Y_D) + (C_B M_I W_D Y_D) \end{bmatrix}\right) + \left(V_L F_I \begin{bmatrix} (C_A M_L W_L Y_L) + (C_B M_L W_I Y_L) \\ + (C_A M_I W_I Y_L) + (C_B M_I W_L Y_L) \end{bmatrix}\right) + \left(V_I F_I \begin{bmatrix} (C_A M_I W_I Y_I) + (C_B M_I W_L Y_I) + (C_B M_I W_D Y_I) \\ + (C_A M_L W_L Y_I) + (C_B M_L W_I Y_I) + (C_B M_L W_D Y_I) \\ + (C_A M_D W_D Y_I) + (C_B M_D W_L Y_I) + (C_B M_D W_I Y_I) \end{bmatrix}\right)$$

The fitness for Light and Intermediate individuals under Pair with Own Phenotype Strategy can be shown as:

$$L_O = \left(V_L F_L \left[\left(C_A W_L Y_L\right) + \left(C_B W_I Y_L\right)\right]\right) + \left(V_I F_L \left[\left(C_B W_I Y_I\right) + \left(C_B W_D Y_I\right)\right]\right)$$

$$I_O = \left(V_I F_I \left[\left(C_A W_I Y_I\right) + \left(C_B W_L Y_I\right) + \left(C_B W_I Y_I\right)\right]\right) + \left(V_L F_I \left[\left(C_B W_L Y_L\right) + \left(C_A W_I Y_L\right)\right]\right) + \left(V_D F_I \left[\left(C_B W_D Y_D\right) + \left(C_A W_I Y_D\right)\right]\right)$$

Chapter 4

LEVELS OF INBREEDING IN THE COLOUR POLYMORPHIC COMMON BUZZARD

This chapter forms the basis of paper in preparation as Fowlie, M.K. & Amos, W. Levels of inbreeding in the colour polymorphic common buzzard (*Buteo buteo*).

ABSTRACT

The detrimental effects of inbreeding are well known, and they have been shown to be associated with lower levels of reproductive success, higher levels of parasitism and differences in disease susceptibility. To better understand large fitness differences between morphs in the colour polymorphic common buzzard, *Buteo buteo*, we investigated differences in the levels of internal relatedness between morphs. As the common buzzard mating system is non-random and the light and dark morph individuals are less abundant than the intermediates, it could be the case the extreme colour morph individuals are more inbred. However, no differences were found in levels of inbreeding. We conclude that inbreeding depression does not explain differences in lifetime reproductive success in the common buzzard.

INTRODUCTION

When close relatives mate, their offspring frequently suffer from reduced fitness. This phenomenon is known as inbreeding depression. The detrimental effects of levels of inbreeding on traits relating to individual fitness have been studied for several decades (Allendorf & Leary 1986; Duarte et al. 1996; Keller & Waller 2002). Inbreeding depression reflects the consequences of increased homozygosity for alleles that affect fitness. It occurs through the superior performance of heterozygote genotypes (heterozygote advantage) and/or the build up of deleterious recessive alleles (Charlesworth & Charlesworth 1987, 1999). Evidence exists to support both these mechanisms for inbreeding depression, however partially recessive deleterious mutations explain most cases observed in *Drosophila* (Charlesworth & Charlesworth 1999).

There is evidence for inbreeding depression in several captive vertebrate populations (Lacy et al. 1993) and it has been documented in a number of natural populations too, including invertebrates (Chen 1993; Saccheri et al. 1998), reptiles (Madsen et al. 1996; Olsson et al. 1996), birds (Bensch et al. 1994; Keller et al. 1994; Keller 1998), and mammals (Coltman et al. 1998; Coulson et al.1998). Typically, the effects found are reduced survival and fecundity.

Costs incurred through inbreeding appear to be great enough that individuals have evolved mechanisms by which to avoid them. These include mate choice

based on kinship related cues (Pusey and Wolf 1996; Bull and Cooper 1999), divorce (Hatchwell et al. 2000) and sex biased dispersal (Pusey 1987). Very small populations where inbreeding avoidance is not always possible can even be at risk of extinction due to inbreeding depression (Saccheri et al. 1998).

There is some theoretical evidence that inbreeding is more likely in structured or mate choice limited populations compared to situations where individuals mix more freely (O'Donald 1960; Balloux, Amos & Coulson unpublished manuscript).

In the colour polymorphic common buzzard (*Buteo buteo*) there are three morphs: light, intermediate and dark. Mating in the common buzzard has been shown to be non-random (Krüger et al. 2001) meaning that panmictic mixing of genes is unlikely. Birds preferentially pair with individuals of the same phenotype as their mothers, and this is potentially maintained by imprinting on the mother's phenotype (Krüger et al. 2001). O'Donald (1960) suggested that this type of imprinting might result in different levels of inbreeding between morphs. The polymorphism appears to be maintained genetically, controlled by a single locus, with intermediates being heterozygous (Krüger et al. 2001). In addition the morphs have markedly different lifetime reproductive success (LRS). Intermediate birds are almost twice as successful as the other two morphs (Krüger & Lindström 2001). However the exact reasons for these life-history differences between morphs are not clear. Morphs differ in their levels of aggression (Chapter 5) and their timing of breeding (Chapter6), the intermediate birds occupy better breeding territories (Krüger 2002), and the

predation risk by goshawk is heaviest to the light morph (Krüger 2002). However, it is unlikely that any of these factors provide a sufficient explanation for the large fitness differences between the morphs, especially because they do not affect the morphs in a similar way. One further possibility for the poor performance of light and dark individuals is that they are more inbred than the intermediate individuals. Here we quantify the degree of inbreeding in the different morphs of the common buzzard.

MATERIALS AND METHODS

A buzzard population comprising of 35 to 101 breeding pairs per year has been monitored continuously since 1989 in a 300-km^2 are in Eastern Westphalia, Germany.

During the 2001 and 2002 breeding seasons, nests were climbed during the chick rearing stage, blood samples were taken from the brachial vein and individuals were assigned a morph. Adults were caught using mist nets.

DNA Extraction

DNA from the blood was extracted using an ammonium acetate method. Fifteen µl of a blood/lysis buffer mixture was digested at 37°C overnight in 250 µl of digestion buffer (20mM EDTA, 50 mM Tris, 120 mM NaCl, 1% SDS, pH 8.0) with 50 µg of Proteinase K. After digestion, an equal volume of 4 M

ammonium acetate was added, the mixture was vortexed and then left at room temperature for 25 minutes. Then samples were then spun at 13000 rpm for 15 minutes and the pellet discarded. Two volumes of 100% ethanol were added to the supernatant, the mixture was vortexed and then spun at 13000 rpm for 20 minutes. The supernatant was discarded and the pellet washed in 70% ethanol and then air-dried for 30 min. The DNA pellet was dissolved at 65°C for about an hour in 50 – 150 µl of low EDTA TE buffer, depending on the size of the pellet.

The primers used were specifically designed for buzzards. The 11 primers used for the analysis were Bbu03, Bbu10, Bbu11, Bbu14, Bbu16, Bbu17, Bbu22, Bbu30, Bbu35, Bbu42, and Bbu46.

The PCR was performed on a Hybaid PCR machine. The thermal profile for the PCR consisted of an initial step at 94°C for 2 minutes, followed by a cycle of 94°C for 45s, 48°C for 45s, 72°C for 50s, with 12 repeats, then a cycle of 89°C for 30s, 48°C for 45s, 72 for 50s with 22 repeats. The final cycle had an extended period of 5 minutes at 72°C.

0.005 µl of radioactive P^{32} was added to each product. Products were run on acrylamide gels for up to 3 hours depending on the size of product. Gels were developed using a phosphoroimaging machine. Gels were scored blind with respect to individual and morph.

Estimation of parental Similarity

Internal relatedness (IR) is a measure of inbreeding based on allele sharing where the frequency of every allele counts towards the final score, thereby allowing the sharing of rare alleles to be weighted more than the sharing of common alleles. This method was developed by Queller & Goodnight (1989) based on genetic correlations between two individuals but has been applied to data in which, at each locus, two alleles rather than two pairs are compared (Amos et al. 2001)

The basic formula simplifies to:

$$\frac{(2H - \sum_{fi})}{(2N - \sum_{fi})},$$

where, H is the number of loci that are homozygous, N is the number of loci and f_i is the frequency of the ith allele contained in the genotype.

When calculated over several loci the values are approximately normally distributed and centred more or less on zero for individuals born to 'unrelated' parents, with negative values suggesting relatively 'outbred' individuals and high positive values being suggestive of inbreeding.

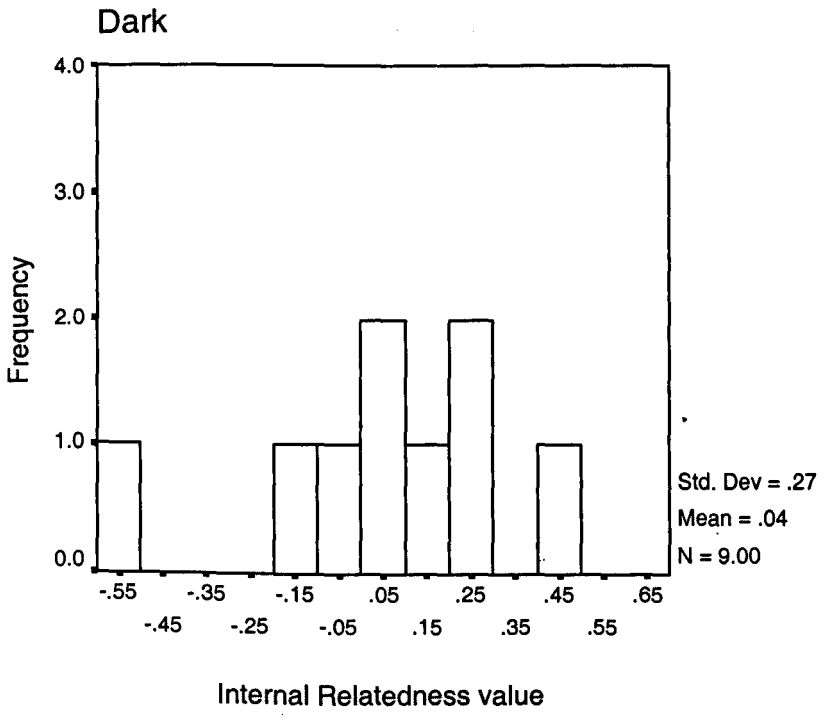
Parental similarities were calculated using an EXCEL macro written in VISUAL BASIC. An overall IR value was calculated and then a jack-knife approach was used removing each locus sequentially to examine single locus effects. ANOVAs were performed on the resulting data using MINITAB 12.1 (Minitab Inc, 1998).

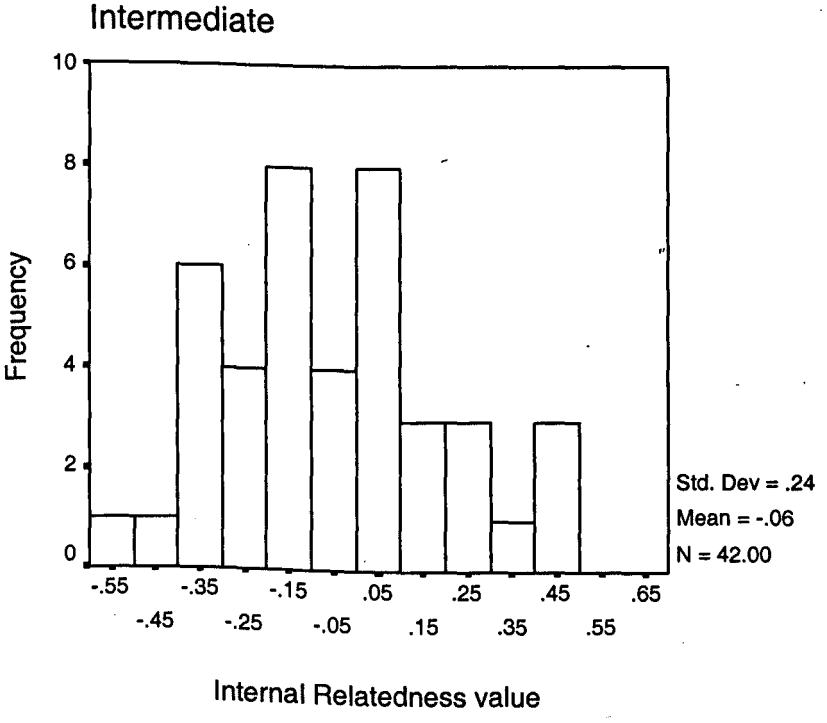
RESULTS

Distributions of IR values were normally distributed within each morph (Fig. 4.1)

There was no difference between colour morphs with respect to their overall IR values ($f = 0.70$; $d.f. = 2$; $p = 0.501$) (Fig. 4.2).

The jack-knifing approach removing each locus sequentially showed no single locus effects on IR values (Fig 4.3). However the locus Bbu17 had a slightly stronger effect than the other loci ($f = 1.55$; $d.f. = 2$; $p = 0.218$)


Dark
Frequency
4.0
3.0
2.0
1.0
0.0
-.55
-.45
-.35
-.25
-.15
-.05
.05
.15
.25
.35
.45
.55
.65
Std. Dev = .27
Mean = .04
N = 9.00
Internal Relatedness value


Intermediate
Frequency
10
8
6
4
2
0
-.55
-.45
-.35
-.25
-.15
-.05
.05
.15
.25
.35
.45
.55
.65
Std. Dev = .24
Mean = -.06
N = 42.00
Internal Relatedness value

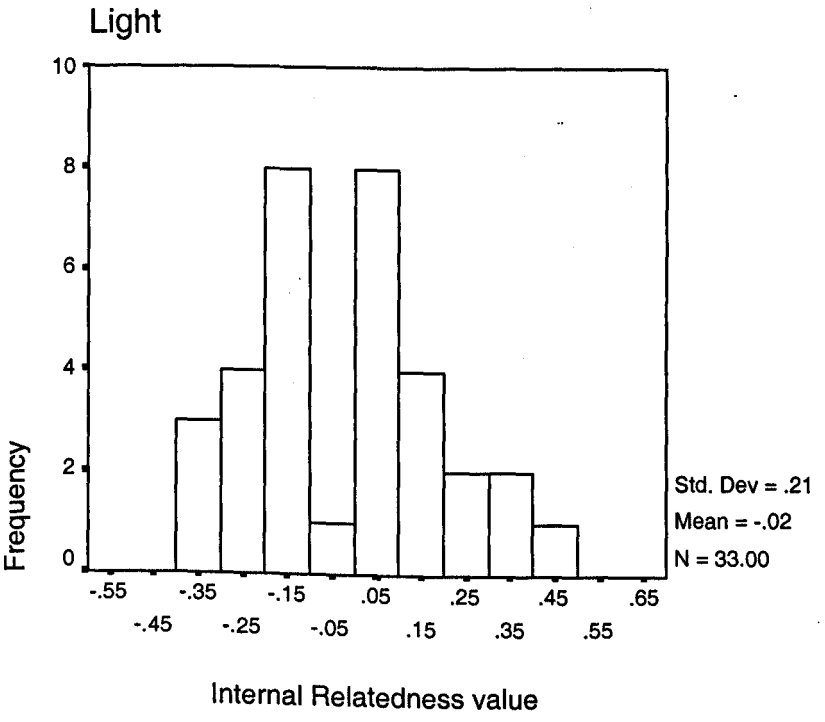


Figure 4.1: The distribution of IR values for each morph

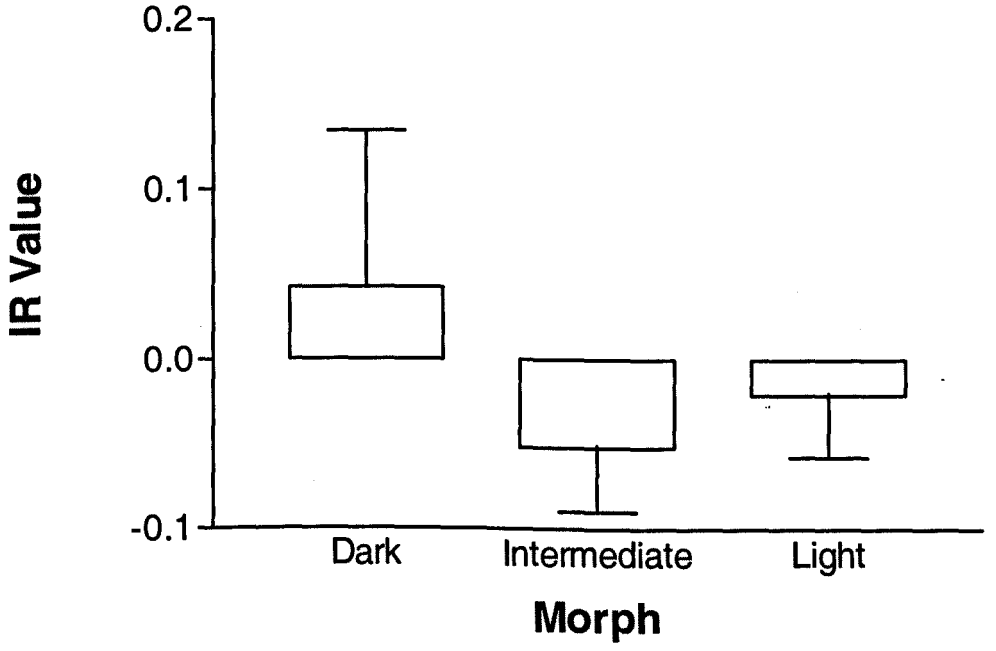


Figure 4.2: Mean Internal relatedness values (IR) between morphs (±1 S.E.)

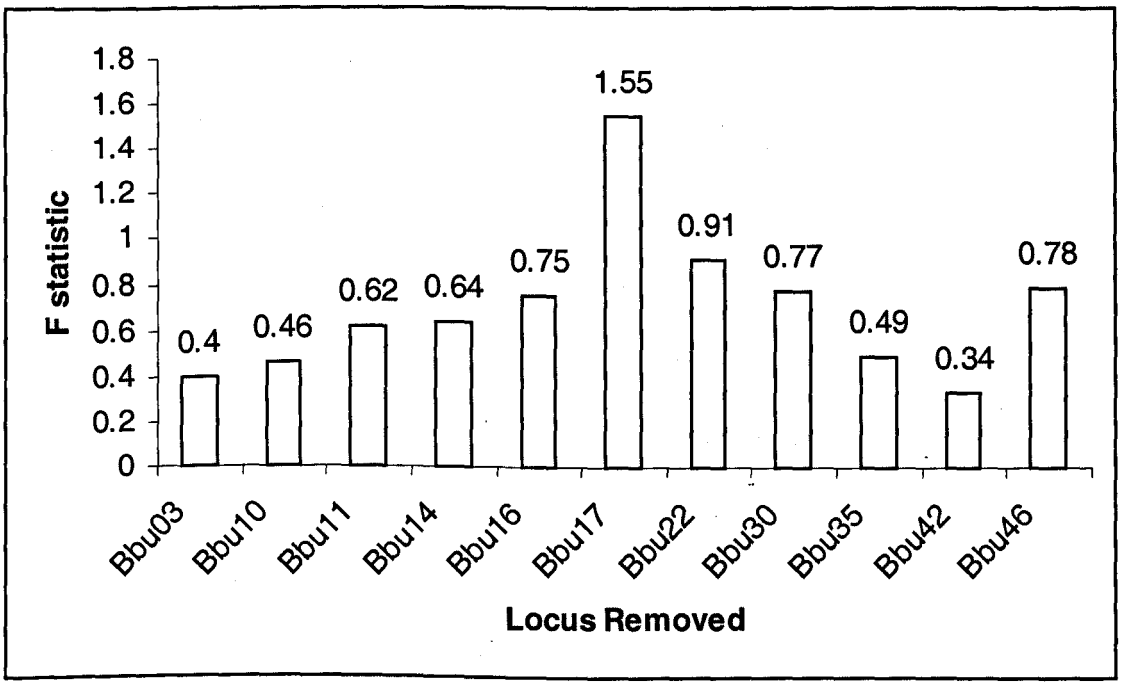


Figure 4.3: *F* statistic with each sequential locus removal and IR value

DISCUSSION

In a non-random mating system, such as the one described in the common buzzard (Krüger et al 2001), one might expect that the less numerous types (like the morphs in buzzards) can be relatively more inbred than the individuals belonging to the majority. In the common buzzards the dark and light morphs are less abundant than the intermediates (10, 30 and 60%, respectively). Here we analysed levels of internal relatedness using polymorphic microsatellites. We found no difference in the levels of inbreeding between the morphs.

This result therefore lends further support to the earlier observations that assortative mating within the colour morphs is either not very strong, or as suggested (Krüger et al 2001) is not the main cue at all that the individuals use. If, as modelling results show, they use mother's phenotype as a basis of mate choice, the result of finding no difference in the levels of inbreeding between the morphs is understandable: most individuals in the population have an intermediate mother. This leads to a very efficient and continuous gene flow between the extreme colour morphs and the intermediates.

Dispersal distances for juveniles in the study population are not known. However, ringing recoveries in Great Britain and Germany suggest that the majority of individuals remain within 50 km of their natal territory (Cramp and Simmons 1980). While this distance is not great, this, coupled with an almost continuous distribution throughout Europe may mean that close relatives are

unlikely to breed together. It is also possible that the extreme colour morph individuals have to disperse on average further than the intermediates as it has been shown (Krüger and Lindström 2001) that the intermediates occupy better breeding territories. It is to be expected therefore that they are better in intra-specific competition and can take over a breeding territory more easily than the light and dark individuals.

Some species of birds have been shown to possess mechanisms of kin recognition (Hatchwell et al. 2001), which are assumed to have evolved for the purpose of inbreeding avoidance. Whether buzzards possess these mechanisms is not known. However, given that individuals appear to imprint on their mother's phenotype, an image based recognition system seems unlikely.

Previous studies have shown a relationship between levels of inbreeding and differences in reproductive success (Keller 1998; Slate et al. 2000). Buzzard morphs show a marked difference in levels of reproductive success, however differing levels of inbreeding appear not to account for these observed differences.

It appears that the mating strategy, possibly coupled with dispersal, prevents differing levels of inbreeding between morphs. This is despite buzzards occurring in a structured population and also possessing a possible imprinting mechanism.

Chapter 5

MORPH SPECIFIC AGGRESSION IN THE COMMON BUZZARD

This chapter forms the basis of a paper in preparation as Fowlie, M.K., Krüger, O. & Russell, A. Morph specific aggression in the Common Buzzard (*Buteo buteo*).

ABSTRACT

In birds, polymorphisms tend to be genetically determined and colour-related, but the physiological and behavioural consequences of such polymorphisms are not widely known. Throughout much of Europe, the common buzzard (*Buteo buteo*) is found in three distinct colour morphs, light, intermediate and dark. We have previously shown that this polymorphism has a genetic basis and that intermediate morphs have significantly higher reproductive success than either of the other two colour morphs. Here we use an experiment to investigate the effect of this melanin-based polymorphism on nest defence behaviour in the common buzzard (*Buteo buteo*). Among males, light morphs were found to be significantly more aggressive to a perceived threat of nest predation than either intermediate or darkly coloured birds, while there was a non-significant tendency for the reverse among females. The level of aggression observed for each member of a pair was independent of the level aggression shown by the other member. These results illustrate that polymorphisms can be associated with alternative reproductive tactics in birds, and suggest a possible link between the biochemistry of melanin production and individual behaviour.

INTRODUCTION

Polymorphisms, in which individuals of the same sex and species show two or more distinct size or colour forms in the same population, occur at low levels in a wide variety of animal taxa (insects, Majerus 1998; fish, Regan 1961, birds, Theron et al. 2001 and mammals, Ritland et al. 2001). Polymorphisms may arise as a consequence of non-genetic and genetic factors and may have dramatic consequences for reproductive behaviour.

Many polymorphisms result from a genetically monomorphic, conditional strategy where high-status individuals become the dominant morph and low-status individuals the subordinate morph (Gross 1996) or are environmentally controlled (Horth 2003). However polymorphisms may be condition independent and genetically controlled, in which individuals are predetermined to become a particular morph (Tsubaki et al. 1997).

In birds, polymorphisms tend to have a genetic basis and often involve differences in colour, but our knowledge of the physiological and behavioural repercussions of such differences is limited. The two best known examples are the ruff (*Philomachus pugnax*) (Lank et al. 1995) and the white-throated sparrow (*Zonotrichia albicolls*) (Tuttle 2003). In the ruff the colour polymorphism is associated with different reproductive tactics, where males either employ independent or satellite tactics at lekking sites (Lank et al. 1995). White-throated sparrows occur in two colour morphs having either a white or tan eyebrow. The morphs are caused by a chromosomal inversion (Thorneycroft 1966). Differences in male behaviour has been observed

between morphs, with white males being more aggressive, giving less parental care and spending less time mate guarding (Tuttle 2003).

A potential third example exists where morphs exhibit different behaviours. Throughout much of its range, the Common Buzzard occurs in three colour morphs: light, intermediate and dark, although there is some variation within each of these categories. The polymorphism appears to be maintained genetically being controlled by a single locus, with intermediates being heterozygous (Krüger *et al.* 2001). In addition, the morphs have markedly different lifetime reproductive success. Intermediate birds are almost twice as successful as the other two morphs, and light birds do considerably better than dark individuals (Krüger & Lindström 2001). The reasons for this extreme trichotomy in reproductive success and longevity are not known. Using the behavioural response to a perceived predator, we test the novel hypothesis that aggressiveness, a trait known to be under the influence of circulatory testosterone levels, differs among colour morphs.

MATERIALS AND METHODS

A buzzard population comprising between 35 to 101 breeding pairs per year has been monitored since 1989 in a 300km^2 area in Eastern Westphalia, Germany. Breeding performance and reproductive state were assessed by repeated visits to each nest. In the breeding seasons of 2001-2003, we carried out an experiment to test the level of aggressive response by light, intermediate and dark individuals to a perceived predator.

This experiment was conducted by placing a stuffed Eagle Owl (*Bubo bubo*) at the forest edge within 50 metres of a nest (n = 53) during the chick-rearing period; eagle owls are known to present a significant threat to young and adult buzzards (Krüger pers. comm.). Once one of the pair was observed to have seen the owl, i.e. by circling above the owl or by alarm calling, an hour was given for the pair to mount a defensive response. Birds were watched from a distance of roughly 50 metres by hidden observers using binoculars.

The response was categorised in to three differing behaviours. A score of 1 was given if an individual did nothing more than circle above the owl and alarm call. A score of 2 was assigned if an individual made some attempt to attack but did not make contact with the owl. If the buzzard made contact then it was given a score of 3.

The sex of the buzzards at each nest were assigned using morphological (females are on average 5-10% larger and heavier than males), behavioural (females are responsible for almost all incubating) and genetic data. The number of chicks in each nest were counted and their age at the time of the experiment was calculated by back dating from their eventual date of fledging.

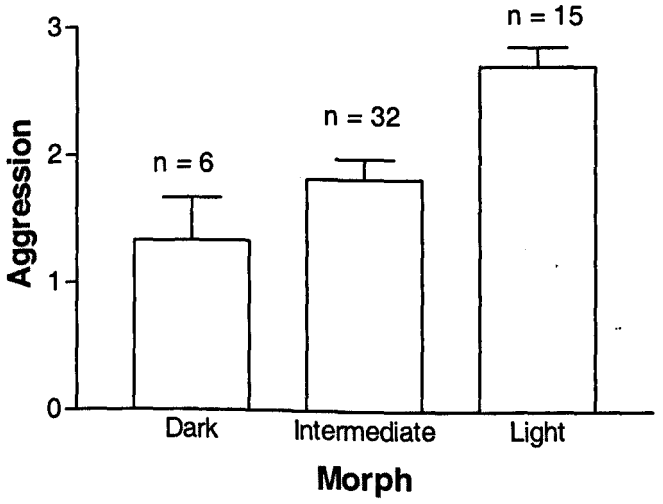
Data were analysed using two Ordinal Logistic regressions (one for each sex) in Minitab 12.1 (Mintitab Inc, 1998). In each analysis, level of aggression was fitted as the response term, while individual morph was fitted as the primary explanatory factor. In each analysis, we also controlled for the age and number of chicks, as well as the level of aggression shown by an individual's partner. However, these potential

confounding factors were dropped from models if their p value exceeded 0.1, indicating they explained a non-significant proportion of the total variation. Finally, using Fisher's Exact test, we also examined the level of response within intermediate coloured birds, to determine whether those lighter than average were more or less aggressive than those darker on average. In this case, we combined aggression levels one and two and compared these with three.

RESULTS

Male morph had significant effect on aggression ($G = 15.82$; $d.f. = 2$; $p < 0.001$. Goodness of fit deviance $\chi^2 = 63.19$; $p = 0.94$). Light morphs were significantly more aggressive than either dark morphs ($z = -2.95$, $p = 0.003$) or intermediates morphs ($z = -2.68$, $p = 0.007$). There was no difference in aggression between dark and intermediate birds ($z = -1.51$, $p = 0.13$). (Fig. 5.1a). Chick age and chick number had significant or near significant effects on overall male aggression ($z = 2.00$; $p = 0.046$, and $z = 1.87$; $p = 0.061$ respectively). In contrast, there was no association between the level of aggression shown by a male and that shown by its partners."

(*a*)



(*b*)

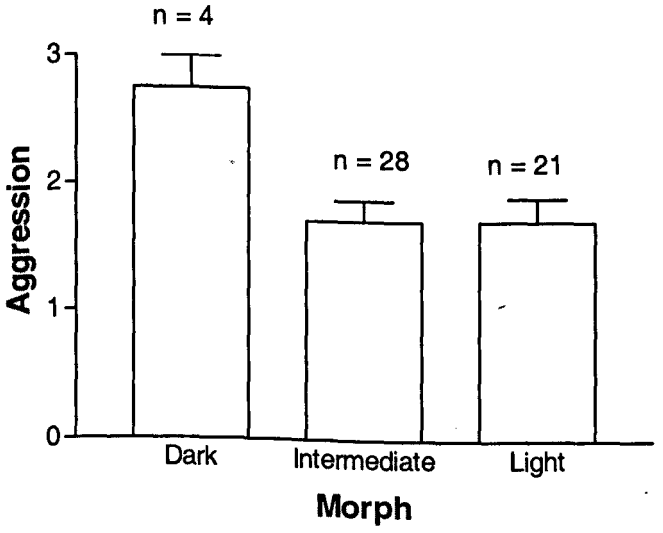
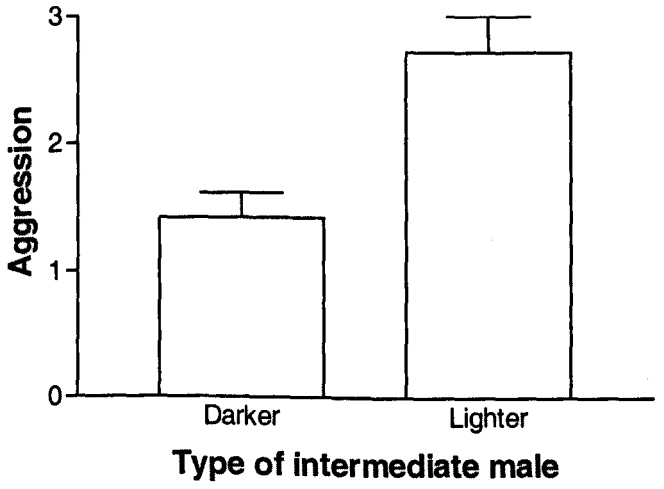


Figure 5.1: (*a*) Mean aggression for male morphs (±S.E.). (*b*) Mean aggression for female morph (±S.E.).

a)



b)

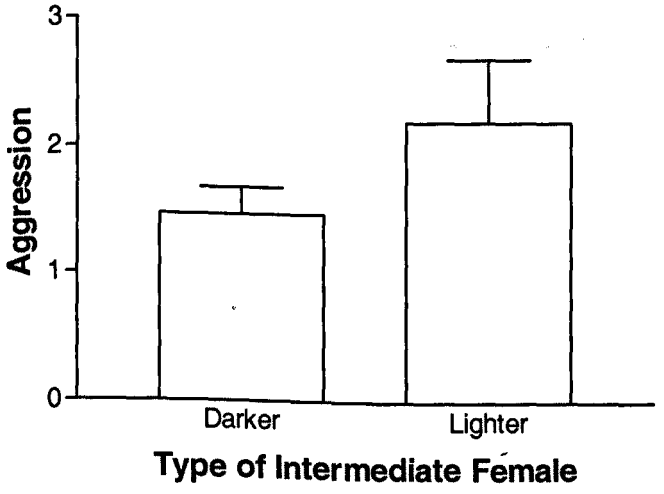


Figure 5.2: (*a*) Mean aggressive response of darker intermediate males versus lighter intermediates. Lighter individuals were significantly more aggressive ($p = 0.005$) and (*b*) Mean aggressive response of darker intermediate females versus lighter intermediates. There was no difference between them ($p > 0.05$).

Overall, female morph had no effect on aggression ($G = 1.09$; *d.f.* = 2; $p > 0.5$. Goodness of fit deviance $\chi^2 = 1.14$; $p = 0.57$). However, in contrast to males, there

was a strong tendency for dark morphs to be more aggressive than either light morphs ($z = 1.88$; $p = 0.060$) or intermediate morphs ($z = 1.92$; $p = 0.055$). (Fig 5.1b). The aggression levels of females were unaffected by chick age ($z = 0.21$; $p =$ 0.0.83), number ($z = 1.01$; $p = 0.31$) or the level of aggression shown by their partner ($z = -1.142$; $p = 0.15$).

Finally, differences in morph-related levels of male aggression were also mirrored within the intermediate category, with lighter than average intermediates being more aggressive than darker than average intermediates (Fisher exact test, $p = 0.005$). (Fig. 5.2a). However, this was not the case among females, although the number of darker than average birds was small in this case (Fisher exact test, p = 0.98). (Fig. 2b).

DISCUSSION

Here we show that for male Common Buzzards morph explains differences in aggression between light individuals and the other two morphs. Within the intermediate morph the extent of melanin pigmentation also explains the variation in aggressive behaviour. Also chick age at the time of the experiment and chick number had slight effects on aggression.

A slight effect of chick age and chick number is to be expected, as the investment that individuals have given to that given breeding attempt is greater and so worth greater defence.

For females there was no significant difference in aggression between morphs although there was a strong tendency for dark females to be more aggressive. Chick number, age and the aggressive response had no effect on behaviour.
For both sexes sample sizes of dark individuals were very low due to the low levels of recruitment and the poor chances of dark individuals reaching the chick rearing stage.

For males, observations suggest that dark morphs are the least aggressive with the reverse being true for females.

In vertebrates, differing levels of melanin deposition in plumage, pelage or skin is predominantly responsible for the observed colour morphs. Animals manufacture melanins from amino acid pre-cursors with special pigment cells called melanocytes (Jawor & Breitwisch 2003). These amino acid pre-cursors are also utilised in a wide variety of other biochemical pathways and it is unclear whether there is a trade-off in melanin utilisation between integument and other biochemical processes.

Several studies have examined melanin based sexual ornaments and their consequence in mate choice, lifetime fitness and behaviour. While sexual ornaments are discrete areas, easily identifiable, some studies have used plumage wide characteristics in their assessment of life history trade-offs (Roulin 1999; Niecke et al. 2003).

Testosterone is an important regulator of melanin pigmentation in feathers, scales, fur and hair and also controls competitive and aggressive behaviour in males (Jawor & Breitwisch 2003). In House sparrows (*Passer domesticus*), the black bib is enlarged by testosterone during the moult (Gonzalez et al. 1999, Evans et al. 2000). However in European Starlings (*Sturnus vulgaris*) testosterone has the reverse effect and high levels inhibit melanin deposition (Witschi and Miller 1938). As starlings have a high concentration of melanin throughout their plumage they are perhaps a better comparison for buzzards than other species. If this is the case for buzzards then light individuals should have high levels of testosterone thereby leading to increased aggression in males in terms of intra-specific interactions and anti-predator defence. This certainly seems to be the case and this result is reinforced by the subtle differences seen within the intermediate morphs, where the lighter birds are more aggressive.

What are the consequences of this potentially large difference in testosterone level? Light morphs by being very aggressive may suffer more risk in terms of interactions with other individuals and predators thereby reducing their longevity. Also, higher levels of testosterone may destabilise pair bonds thereby reducing reproductive output. Light birds may also incur a physiological cost by having high levels of testosterone, as testosterone also acts an immunosuppressant.

For dark males the picture is less clear. If they are indeed less aggressive then this will affect their ability to compete for mates and territories, which will obviously have detrimental effects on their lifetime reproductive success (LRS).

Intermediate birds are the most successful in terms of LRS and may well have achieved a balance between melanin deposition and testosterone, and as a consequence, levels of aggression. A middling level of testosterone would have neither of the adverse effects of the other two morphs.

Buzzard morphs are constrained by their genetics and mate choice (Chapter 3) and this possibly has knock on effects in terms of their biochemistry, leading to the observed differences in lifetime reproductive success. Hormonal testing of individuals of known morph is needed to establish levels of testosterone and its effects on morph behaviour.

Chapter 6

TIMING OF REPRODUCTION VARIES WITH COLOUR MORPH IN THE COMMON BUZZARD.

This chapter forms the basis of a paper submitted as Fowlie, M.K., Lindström, J., Krüger, O. & Russell, A. Morph specific aggression in the Common Buzzard (*Buteo buteo*).

ABSTRACT

For most species living in seasonal environments timing is an important determinant of the success of a breeding attempt. Individuals also face a trade off between current and future reproduction. In the colour polymorphic common buzzard (*Buteo buteo*) there is large variation between morphs in their lifetime reproductive success where the intermediate morph is almost twice as successful as the two extreme morphs. Here we investigated whether colour morphs differed in their timing of breeding. Light-light and dark-dark pairs were found to breed earlier than the population mean, with light-dark pairs fledging chicks slightly later. Differences in reproductive strategies between morphs possibly account for the observed differences.

INTRODUCTION

The seasonal timing of reproduction is a trait that can have large repercussions on lifetime fitness across a wide range of animal taxa (Clutton-Brock 1988; Schultz 1993; Olsson and Shine 1997). By far the largest body of such work has focussed on birds (Nilsson 1999). Generally, earlier breeding is associated with larger clutches, more fledglings, and in the longer term more recruits from first clutches than those individuals breeding later (Perrins 1970; MacColl & Hatchwell 2002). Finally, early breeding may not only be beneficial for offspring, but also for the parents, allowing them a longer post-breeding period in which to replenish resources and enhance survival to the following year (Nilsson and Svensson 1996).

There have been two mechanisms proposed to explain seasonal variation in single fitness components: the date hypothesis and the parental quality hypothesis (Brinkhof et al. 1993; Nilsson 1999). The date hypothesis predicts that the timing of breeding affects all pairs the same with respect to the value of a fitness component. However, individuals will vary in their trade-offs between current and future reproduction and this will, to some extent, be dependent on differences in quality between parents and territories (Nilsson 1999). Therefore individuals may employ different tactics to maximise fitness. The parental quality hypothesis predicts that variation in phenotypic characters of the parents (e.g. age or breeding experience, Sæther 1990) or environmental quality (e.g. the quality of territory, Alatalo et al. 1986) will lead to differences in the timing of breeding.

Throughout much of its range the Common Buzzard (*Buteo buteo*) occurs in three colour morphs: light, intermediate and dark. The polymorphism appears to be maintained genetically, controlled by a single locus, with intermediates being heterozygous (Krüger et al. 2001). In addition the morphs have markedly different lifetime reproductive success (LRS). Intermediate birds are almost twice as successful as the other two morphs (Krüger & Lindström 2001). However the exact reasons for these life-history differences between morphs are not clear. Here we examine whether the colour morph of an individual and/or the pair combination has an effect on the eventual fledgling date of a reproductive attempt, thereby elucidating a potential mechanisms for differences in reproductive success found earlier (Krüger & Lindström 2001).

MATERIALS AND METHODS

A buzzard population comprising 35 to 101 breeding pairs per year has been monitored since 1989 in a 300-km^2 area in Eastern Westphalia, Germany. During each breeding season, repeated visits to each nest established the morph combination of each breeding pair, the number and morph of chicks and their eventual fledging date. Exact hatching dates, clutch size and growth rates of chicks was not known due to nest-site location constraints, and so date of fledging, for first attempts only, was used as a proxy for lay date.

All pairs with successful breeding attempts in the years 1991 to 2003 were used in the analysis (n = 536).

Statistical analyses

The effect of individual morph on fledge date was analysed using a linear mixed model with a normal error structure in GENSTAT 5, release 4.1 (GENSTAT, Rothamstead Experimental Station, Harpenden, UK). A linear mixed model with a normal error is similar to a general linear model except that it allows both fixed and random terms to be fitted (Schall 1991). Random terms take into consideration repeated sampling of the same individual and of different individuals within the same territory. In the analysis, fledge date (calculated as the number of days before or after the mean in each year) was fitted as the response term, while maternal, paternal and territory identities were fitted as random terms. Maternal identity ($p < 0.01$) and paternal identity ($p = 0.1$) were maintained as random terms, but territory identity was dropped as it had a negative component of variance, indicating that it explained none of the variation in the analysis.

In GENSTAT, the significance of explanatory terms in linear mixed models is assessed by their Wald Statistics, which are distributed approximately as χ^2 for each term fitted last in the model.

RESULTS

There are nine potential pairing combinations in the common buzzard, corresponding to the three morph types and whether it is the male or female that carries that certain morph. However, some of these potential categories were combined. First, we combined dark-light and light-dark pairings due to their rare occurrence (n = 4). Second, previous analyses have shown that intermediate-intermediate pairings are most successful, but we have no *a priori* reason for suspecting that the success of mixed pairings containing one intermediately coloured bird and one light/dark bird will vary systematically with respect to the sex of the intermediate or the colour of the other pair member. This notion was confirmed through preliminary analysis that fledge date was unaffected by whether it was the male or female that was intermediate or whether the other member of the pair was dark or light ($\chi^2 = 1.35$, d.f. = 3, $p = 0.72$). We consequently combined all dark-intermediate, intermediate-dark, light-intermediate, and intermediate-light pairings into a single category.

Analysis of the final five morph-pair combinations revealed highly significant differences in fledge date ($\chi^2 = 19.27$, d.f. = 4, $p = 0.001$, Figure 6.1). However, our prediction that the most successful pair combinations (i.e. intermediate-intermediate) would fledge chicks earliest was not upheld. Instead, dark-dark pair combinations fledged their young significantly earlier than light-light combinations and both fledged young significantly earlier than all other pair combinations.

Fledge dates are related to the number of chicks fledging, with single chicks fledging significantly earlier than two chicks, which in turn fledge earlier than three (χ^2 = 33.87, d.f. = 2, $p < 0.001$, Figure 6.2). Dark-dark and light-light pairings fledge less offspring than other combinations ($\chi^2 = 25.18$, d.f. = 4, $p < 0.001$, Figure 6.3). However, these differences are unlikely to fully explain why dark-dark and light-light pairings fledge chicks earlier than other combinations, for colour-morph combination effects remain significant even after fledging number has been controlled for statistically ($\chi^2 = 13.26$, d.f. = 4, $p = 0.010$, Figure 6.4).

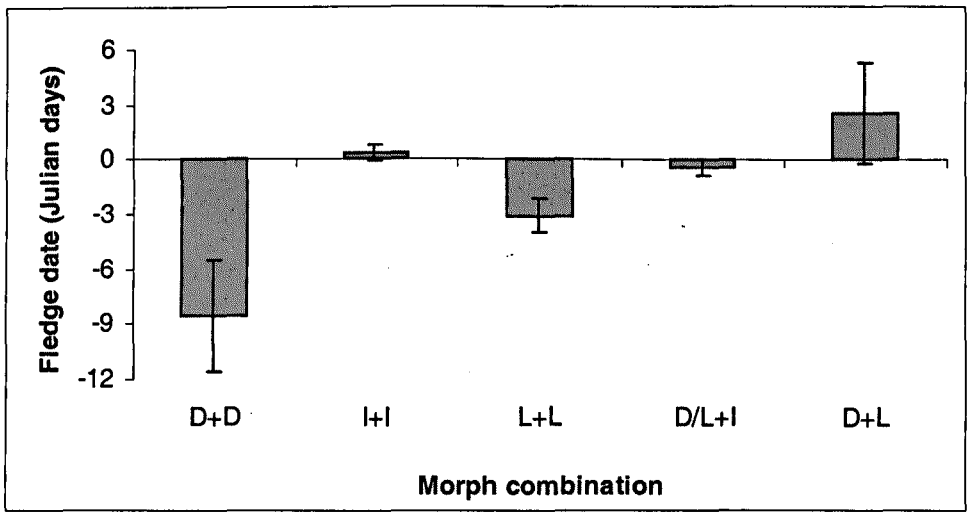


Figure 6.1: The five morph pairings analysed for mean fledging date. Means ± 1 S.E. D refers to dark, L to light and I to intermediate (see also below).

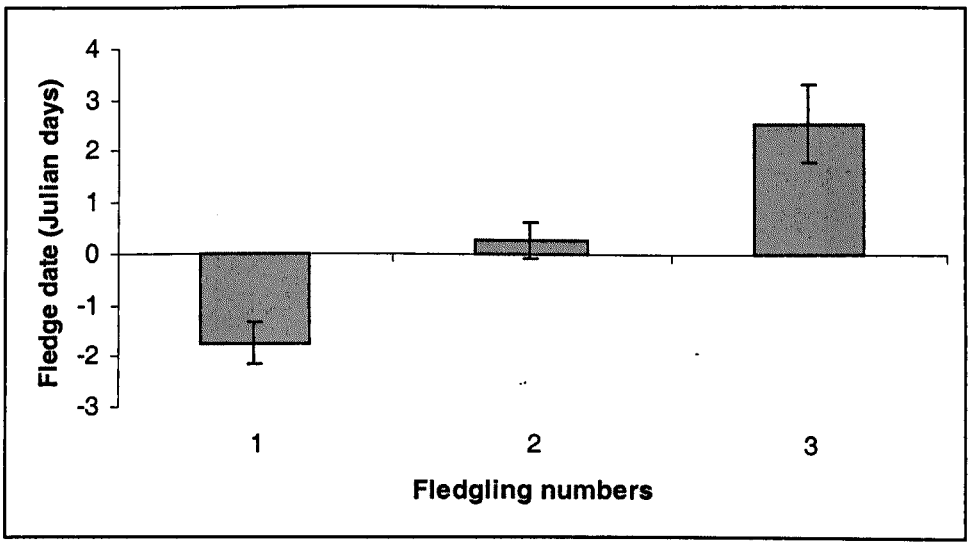


Figure 6.2: Association between chick numbers fledging and fledge date.

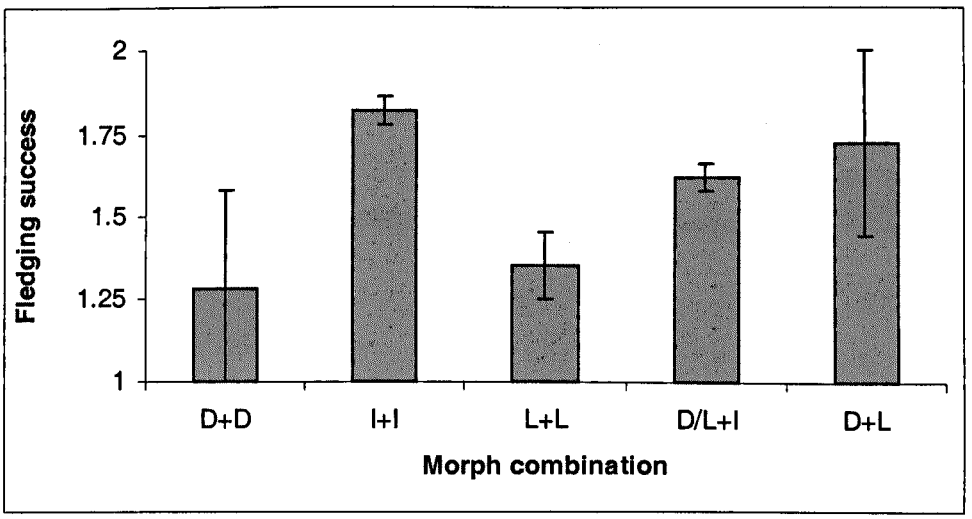


Figure 6.3: Dark/Dark and Light/Light pairings fledge fewer chicks than other morph combinations. Means ± 1 S.E.

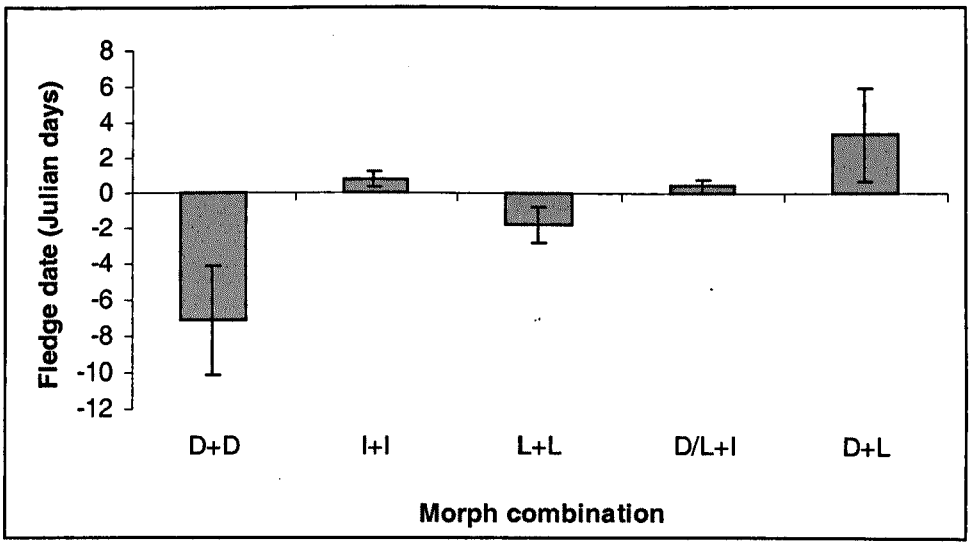


Figure 6.4. After controlling for chick number, extreme morph pairings still show an earlier fledging date.

DISCUSSION

For most species living in seasonal environments, timing of reproduction is very important for breeding success (Daan et al. 1988). In birds, it has been shown that delays in the onset of a reproductive bout often result in lowered breeding success (Daan et al. 1988) and that individuals often assess several cues before commencing a breeding attempt (Svensson 1995). Here we analysed the effects of timing of breeding in the common buzzard. As it has been shown, the three colour morphs have very different LRS (Krüger & Lindström 2001), and it therefore of interest to see whether differences in timing of reproduction possibly contribute to these differences in LRS.

When intermediate morphs breed, there is no significant effect of their partner's morph on the date of chick fledging. In contrast, significant differences in fledge date were observed when either of the two extreme morphs (light and dark) bred with their own morph or that of the other extreme. Light-light and dark-dark pair combinations both fledged young earlier than all other pair combinations, while dark-light pair combinations fledged young at a similar time to intermediate pair combinations.

Light pairs and dark pairs produce fewer fledglings per breeding attempt (Krüger & Lindström 2001, this study). Buzzards' clutch size varies from 1-5, with a three-day delay between the laying of each successive egg in a clutch (Cramp and Simmons 1977). Thus, differences in fledge date may in part be a consequence of differences

in clutch size rather than lay date *per se*. For example, since nest mates fledge at the same time (personal observations), if light-light or dark-dark pair combinations lay smaller clutches than other pair combinations then they will automatically fledge chicks earlier. Differences in clutch size may also lead to further differences in fledge date, since many chicks may grow more slowly than few chicks.

However, differences between clutch sizes and chick growth rates are unlikely to explain fully the differences in morph-related fledge dates observed in this study. This is because, morph-related differences in fledge date remained significant even after controlling for differences in the number of offspring fledging. The question remains, why should dark-dark and light-light pairings fledge chick earlier than any other pair combination? This question is especially interesting given that the extreme morphs have been shown to have a lower lifetime reproductive fitness and have been assumed to be of a lower quality (Krüger & Lindström 2001). By constantly producing smaller clutches light-light and dark-dark morph combinations may be able to attain breeding condition earlier than other pair combinations and thus begin reproduction at an earlier date. This is unlikely however, as it cannot explain why light-dark combinations fledge chicks later.

Interestingly, despite the apparent low quality of light and dark birds (Krüger & Lindström 2001), light - dark pairings appeared to fledge chicks later than other pair combinations, and to have greater success than light-light and dark-dark pairings. These findings have two intriguing implications. First, reproductive success in buzzards in not simply a consequence of parental quality, but also chick quality, with intermediate chicks having greater survival than non-intermediate chicks. Second,

since intermediate offspring have a higher lifetime reproductive fitness they may take longer to raise to fledging. Light – dark pairings produce more chicks than either dark – dark or light – light pairings and such chicks will always be intermediate. Either way, light birds would appear to benefit from mating with dark birds and *vice versa*, in order to produce intermediate offspring. Given this option it would be expected for light – dark pairings to be common, since they will typically produce larger broods than light – light or dark – dark pairings. However, it appears that buzzards are constrained from this option, through their mate recognition system being based on maternal phenotype (see Chapter 3).

What must be remembered however, is that most dark pairs and light pairs never reach the chick stage of reproduction, let alone fledge chicks. Therefore the sample of light-light and dark-dark pairs is probably biased towards to the higher quality individuals.

Another possibility is that different morphs have different moult strategies. Buzzards generally undergo a complete moult each season but some remiges are frequently left unmoulted (Forsman 1999). Light individuals have less melanin in their feathers and this leads to increased abrasion and wear (Bonser 1995). In Ural owls (*Strix uralensis*) there has been shown to be a trade-off between reproductive effort and moult (Pietiäinen 1984) and perhaps this also occurs in buzzards. However more information on differences in moult between morphs is required.

Our results seemingly contradict the hypothesis that higher quality birds breed earlier in the season and attain higher fitness. This idea assumes that, as has been

previously shown, light and dark birds are of lower overall reproductive quality. Our results therefore suggest that early reproduction need not be confined to higher quality individuals, and may be selected among low quality individuals or individuals in circumstances where their chances of success is low. Whether or not individuals are selected to breed early will depend on the trade-off between early reproduction, clutch size and reproductive success. Our results provide no support for the lay-date hypothesis, but lend strong support for a 'parental quality hypothesis'. However, the assumption of existing parental quality hypothesis assumes that early reproduction is associated with high quality, we highlight that this may not always be the case.

Further work is needed in terms of recording clutch size, hatch date and growth curves of each chick morph. These data will lead to a more precise understanding of the different strategies that the different morphs appear to use with respect to the timing of breeding.

Chapter 7

GENERAL DISCUSSION

Evolution and Maintenance of colour polymorphism

Cases of colour polymorphism fall into three broad categories:

1) Examples where a polymorphism is known to exist but no details are known about life history, fitness differences or the mechanism. e.g. Jacobin cuckoo (*Clamator jacobinus*).
2) Cases where species exhibiting colour polymorphism are well studied, however, reasons for the polymorphisms and differences between morphs are not well understood eg. the common guillemot, *Uria aalge* (Jeffries & Parslow 1976; Harris et al. 2003)
3) Finally, examples where differences between morphs are known. These include cases where the genetics of the system are known (Lank et al. 1995; Tuttle 2003). The common buzzard falls into this category as the parent-offspring combinations strongly suggest a simple Mendelian mechanism for the inheritance, and the fitness differences between the morphs have been documented (Krüger et al. 2001).

For colour polymorphism to exist at all it first has to evolve and subsequently has to be maintained in the population. The comparative analysis in Chapter 2 shows that the 'classic' explanation for colour polymorphism in raptors and owls, 'the avoidance image hypothesis' (Paulson 1973; Rohwer & Paulson 1987) doesn't seem to hold true. Population size was shown to be important for the assumed initial mutation to occur but the maintaining mechanisms can not be found with the data available for this analysis. Considering the spread of polymorphism in a population after the initial mutation, a neutral mutation will only spread by genetic drift.

However, this is far less likely in larger populations (Crow and Kimura 1970). It is therefore more likely that either this mutation brings some benefits and spreads through the population, or an "evolutionary trap" may cause the maintenance. A likely example of the latter mechanism is provided by the common buzzard polymorphism: even though the polymorphism is detrimental to two of the morphs, due to the genetic constraints buzzards are unable to "break out". If the buzzards were to escape from this "evolutionary trap" by behavioural means, this would require strict assortative mating with the same phenotype only. However, this would quickly "empty" the pool of intermediate individuals from the population (Jacquard 1974), and would lead to speciation via reproductive isolation. Therefore, the mere existence of all three colour morphs in a population tells us that assortative mate choice with own phenotype only, can not be the case. Thus the idea of an "evolutionary trap" is an intriguing possibility and may also be the case in other species. Other long-term studies are needed to reveal the possible extent of this occurrence.

Mate choice

When the different morphs in a population have different survival probabilities and different fecundities as in common buzzards, mate choice can not be studied in isolation from population demography: mate choice affects the population structure as different mate choice strategies produce different offspring combinations. A demographically explicit population model coupled with an invasion analysis revealed that what had been suggested as being maladaptive (Krüger et al. 2001) was

in fact not. Revealing as this sort of an approach can be, it can not be adopted in a majority of cases as not enough is known about the morphs. One of the only other well-studied systems is that of the side-blotched lizard (*Uta stansburiana*) where there is a three morph mating system. Frequency-dependent selection and the among-year changes in morph fitnesses suggest that male interactions drive a dynamic 'rock-paper-scissors' game (Sinervo & Lively 1996).

Mate choice in the common buzzard is complex and is based on several criteria including phenotype and population structure. Modelling of the mate choice strategies showed that the observed strategy was not the fittest strategy. However genetic constraints mean that they appear to do the 'best of bad job'.

This result has important implications for our understanding of the adaptiveness of individual choice and the evolution of mating systems. What at first appears maladaptive (Krüger et al. 2001) is in fact not when mate choice is studied in a demographic context. Caution is warranted when assessing the adaptiveness of mate choice strategies that have the potential to affect population demography if the consequences to population structure are not taken into account.

Behavioural and fitness consequences of colour polymorphism

Levels of inbreeding have been shown not to differ between morphs and hence are not a factor in differences in lifetime reproductive success between colour morphs (Chapter 4). Other studies have found a strong association between inbreeding and differences in reproductive success (Slate et al. 2000), parasite load (Coltman et al.

1999) and disease susceptibility (Acevedo-Whitehouse et al. 2003). However despite assortative mating and possible imprinting the extreme morphs do not suffer from higher levels of inbreeding.

In Chapter 6, I showed that different morph pairings exhibit differences in the timing of reproduction. Different morphs may employ different strategies for reproduction to solve the trade off between current and future reproduction. Differing strategies may explain the lower chick number fledged by the extreme morphs. However, more information on parental care and clutch sizes is needed to clarify this.

In Chapter 5, I examined differences in aggression between morphs. These differences are perhaps a direct consequence of colour *per se*. Therefore, colour can be associated with long term effects on an individual's longevity either through increased competitive interactions and/or by physiological mechanisms. Intermediates appear to have the balance of testosterone correct and this may be a factor towards their superior performance. Darks are the least aggressive and are possibly inferior in intra-specific competition for territory and mates, whereas lights are highly aggressive but may suffer from higher predation by goshawks (*Accipiter gentilis*) (Krüger 2002). Other examples of behavioural traits associated with colour morph are found in the side-blotched lizard (*Uta stansburiana*), where males of different morph have different mating strategies (Sinervo and Lively 1996) and in the damselfly (*Mnais costalis*) where males employ either 'fighter' or 'sneaker' tactics (Tsubaki et al.1997; Plaistow & Tsubaki 2000).

Future Work

Even though the buzzard data set forming the basis of this work is rather extensive, there are gaps in it that leave ample space for future work. Individual growth curves for morphs throughout development would be necessary to assess differences in growth. Morphs may exhibit different growth strategies that have consequences later in life. Females may also differentially allocate food depending on the offspring morph combination in a nest, or parents of different morphs may differ in their parental ability.

We currently do not know the recruitment rate of individuals in the study population. The colour ringing of birds which is now underway will over time lead to a better understanding of where fledglings from the study site go. This will help further in addressing the questions about the proximate mechanisms causing the fitness differences between the morphs.

To ascertain with certainty the mating strategy that common buzzards use, and to find out for instance whether different morphs are truly genetically of different quality, a series of cross-fostering experiments could be carried out to examine mate choice. However, this is problematic for two reasons. Firstly, to have a large enough sample size for all three morphs the number of chicks needed to be swapped would be in the hundreds and the logistics involved would be enormous. Secondly, if pairing with mother's phenotype is indeed what buzzards use then cross-fostering

experiments would change the direction of evolution of mate choice and demography of the population and for ethical reasons this would be unacceptable.

One of the major assumptions in this work has been the negligible level of extra-pair copulations in the buzzard. Other raptors are known to have low levels (Newton 1979) and it has been assumed that buzzards do not differ. However the means to fully test this assumption are now available through microsatellite genotyping of both chicks and adults. For all nests monitored only a tiny fraction of offspring have been produced that are the 'wrong' morph for the parents that are producing them (Fig 7.1). This may suggest that occasionally individuals are unfaithful to their social partner. If this is the case one would expect female buzzards to seek extra-pair copulations with those males that would maximise intermediate offspring production. However, as with mate choice individuals may be constrained from this by imprinting.

Table 7.1: Parent-offspring phenotype frequencies for all successful broods. Mean observed morph frequencies (%) and predicted means based on Mendelian segregation (in parentheses).

Morph combination of parents	N	Offspring morph frequencies (%)		
		D	I	L
D*D	2	100 (100)	0 (0)	0 (0)
D*I	71	43.7 (50)	56.3 (50)	0 (0)
D*L	8	0 (0)	100 (100)	0 (0)
I*I	294	17.0 (25)	66 (50)	17.0 (25)
I*L	159	1.3 (0)	48.4 (50)	50.3 (50)
L*L	33	0 (0)	0 (0)	100 (100)

In Great Britain colour morphs in the common buzzard are entirely absent or at least occur at such low levels as to be negligible. Only the intermediate morph occurs. This raises the interesting possibility that the UK population has somehow managed to overcome the 'evolutionary trap' that the mainland European population is in.

The most likely explanation is that the gene for colour has been duplicated at least once (Dulai et al. 1999). This then would mean that homozygotes would occur only very rarely. Once the area of the genome that codes for colour is established then comparisons between populations can be carried out to see if this is true. A measure of gene flow through Europe would also allow us to speculate on whether this phenomenon may spread to almost eliminate colour polymorphism over time. A better understanding of gene flow would also allow us to assess the extent to which the UK population is reproductively isolated.

Finally, Theron et al. (2001) have recently found the genetic basis of the melanic form of bananaquit (*Coereba flaveola*), it will be interesting to discover if differences in the melanocortin-1 receptor are also responsible for the morphs present in the common buzzard.

Literature Cited

Acevedo-Whitehouse, K., Gulland, F., Grieg, D. & Amos, W. 2003. Inbreeding: Disease susceptibility in California sealions. *Nature.* **422**, 35.

Alatalo, R.V., Lundberg, A. & Glynn, C. 1986. Female pied flycatchers choose territory quality and not male characteristics. *Nature.* **323**, 152-153.

Allendorf, F.W. & Leary, R.F. 1986. Heterozygosity and fitness in natural populations of animals. In: *Conservation Biology* (ed. Soule, M.E.), pp. 57-76. Sinauer, Sunderland, MA.

Allison, A.C. 1955. Aspects of polymorphism in man. *Cold Spring Harbor Symp.Quant.Biol.* **20**, 139-149.

Allison, A.C. 1964. Polymorphism and natural selection in human populations. *Cold Spring Harbor Symp.Quant.Biol.* **29**, 139-149.

Amos, W., Worthington Wilmer, J., Fullard, K., Burg, T.M., Croxall, J.P., Bloch, D. & Coulson, T.N. 2001. The influence of parental relatedness on reproductive success. *Proceedings of the Royal Society of London Series B-Biological Sciences* **268**, 2021-2027.

Andersson, M.B. 1994. *Sexual selection*, 1st edn. Princeton.

Ayala, F.J. 1976. *Molecular Evolution.* Sunderland, Massachusetts.

Barraclough, T. G., Harvey, P. H. & Nee, S. 1995. Sexual selection and taxonomic

diversity in passerine birds. *Proceedings of the Royal Society of London Series B* **259**, 211-215.

Bensch, S., Hasselquist, D., Nielsen, B. & Hansson, B. 1994. Genetic similarity between parents predicts hatching failure: non-incestuous inbreeding in the great reed warbler? *Evolution* **48**, 317-326.

Bonser, R. 1995. Melanin and the abrasion resistance of feathers. *Condor* **97**, 590-591.

Brinkhof, M.W.G., Cave, A.J., Hage, F.J. & Verhulst, S. 1993. Timing of reproduction and fledgling success in the coot *Fulica atra*: evidence for a causal relationship. *Journal of Animal Ecology* **62**, 577-587.

Brommer, J., Kokko, H. & Pietiainen, H. 2000. Reproductive effort and reproductive values in periodic environments. *American Naturalist* **155**, 454-472.

Brooks, R. 2000. Negative genetic correlation between male sexual attractiveness and survival. *Nature* **406**, 67-70.

Brown, J.L. 1997. A theory of mate choice based on heterozygosity. *Behavioral Ecology* **8**, 60-65.

Bull, C.M. & Cooper, S.J.B. 1999. Relatedness and inbreeding avoidance in the lizard *Tiliqua rugosa*. *Behavioral Ecology and Sociobiology* **46**, 367-372.

Caldwell, G.S. 1986. Predation as a selective force on foraging herons: effects of plumage color and flocking. *Auk* **103**, 494-505.

Caswell, H. 2001. *Matrix population models: Construction, analysis and interpretation*. Sinauer, Sunderland, Massachusetts.

Charlesworth, B. & Charlesworth, D. 1999. The genetic basis of inbreeding depression. *Genetic Research* **74**, 329-340.

Charlesworth, D. & Charlesworth, B. 1987. Inbreeding depression and its evolutionary consequences. *Annual Review of Ecology and Systematics* **18**, 237-268.

Chen, X. 1993. Comparison of inbreeding and outbreeding in hermaphroditic *Arianta arbustorum* (L) (land snail). *Heredity* **71**, 456-461.

Clutton-Brock, T.H. 1988. *Reproductive success*. Chicago University Press, Chicago.

Coltman, D.W., Bowen, W.D. & Wright, J.M. 1998. Birth weight and neonatal survival of harbour seal pups are positively correlated with genetic variation measured by microsatellites. *Proceedings of the Royal Society of London Series B-Biological Sciences* **265**, 803-809.

Cook, L.M. 1990. Systematic effects on morph frequency in the polymorphic mangrove snail *Littoraria pallescens*. *Heredity* **65**, 423-427.

Cook, L.M. 1992. The neutral assumption and maintenance of color morph frequency in mangrove snails. *Heredity* **69**, 184-189.

Cooke, F., Mirsky, P.J. & Seiger, M.B. 1972. Color preferences in the lesser snow geese and their possible role in mate selection. *Canadian Journal of Zoology* **50**: 529-536.

Cooke, F., Rockwell, R.F. & Lank, D.B. 1995. *The snow geese of La Perouse Bay: natural selection in the wild.* Oxford University Press, Oxford.

Coulson, T.N., Pemberton, J.M., Albon S.D., Beaumont, M., Marshall, T.C., Slate, J., Guiness, F.E. & Clutton-Brock, T.H. 1998. Microsatellites reveal heterosis in red deer. *Proceedings of the Royal Society of London Series B-Biological Sciences* **265**, 489-495.

Cramp, S. & Simmons, K.E.L. 1980. *Handbook of the Birds of Europe, the Middle East and North Africa.* Oxford University Press, Oxford.

Crow, J.F. & Kimura, M. 1970. *An introduction to population genetics theory.* Harper and Row.

Cunningham, E.J.A. & Russell, A.F. 2000. Egg investment is influenced by male attractiveness in the mallard. Nature. **404**: 74-77.

Daan, S., Dijkstra, C., Drent, R. & Meijer, T. 1988. Food supply and and the annual timing of avian reproduction. *Proceedings of the International Ornithological congress* **XIX**, 392-407.

Darwin, C. 1859. *The origin of species by means of natural selection.* John Murray, London.

del Hoyo, J., Elliot, A. & Sargatal, J. 1994. *Handbook of the birds of the world. Vol. 2. New world vultures to guineafowl.* Lynx Edicions, Barcelona.

Dittrich, W. 1985. Gefiedervariationen beim Mäusebussard (*Buteo buteo*) in Nordbayern. *J.Ornithol* **126**, 93-97

Dobzhansky, T. 1970. *Genetics of the Evolutionary Process*. Columbia University Press, New York.

Doty, G.V. & Welch, A.M. 2001. Advertisement call duration indicates good genes for offspring feeding rate in gray tree frogs (Hyla versicolor). *Behavioural Ecology and Sociobiology* **49**, 150-156.

Duarte, L.C., Bouteiller, C., Fontanillas, P., Petit, E. & Perrin, N. 2003. Inbreeding in the greater white-toothed shrew, *Crocidura russula*. *Evolution* **57**, 638-645.

Endler, J.A. 1980. Natural selection on color patterns in *Poecilia reticulata*. *Evolution* **34**, 76-91.

Endler, J.A. 1983 Natural and sexual selection on color patterns in poeciliid fishes. *Environ.Biol.Fish.* **9**, 173-190.

Endler, J.A. 1987. Predation, light intensity, and courtship behaviour in *Poecilia reticulata* (Pisces: Poeciliidae). *Animal Behaviour* **35**, 1376-1385.

Evans, M.R., Goldsmith, A.R. & Norris, S.R.A. 2000. The effects of testosterone on antibody production and plumage coloration in male House Sparrows (*Passer domesticus*). *Behavioral Ecology and Sociobiology* **47**, 156-163.

Falk, C.T. & Li, C.C. 1969. Negative assortative mating: exact solution to a simple model. *Genetics* **62**, 215-223.

Felsenstein, J. 1985. Phylogenies and the comparative method. *American Naturalist* **125**: 1-15.

Ferguson-Lees, J. & Christie, D. A. 2001. *Raptors of the world.* Christopher Helm, London.

Fisher, R.A. 1930. *The Genetical Theory of Natural Selection.* Clarendon Press, Oxford.

Forsman, D. 1999. *The raptors of Europe and the Middle East.* T & AD Poyser.

Forsman, A. & Shine, R. 1995. The adaptive significance of colour pattern polymorphism in the Australian scincid lizard *Lampropholis delicata. Biological Journal of the Linnaean Society* **55**: 273-291.

Franck, D., Dikomey, M. & Schartl, M. 2001. Selection and the maintenance of a colour pattern polymorphism in the green swordtail (*Xiphophorus helleri*). *Behaviour* **138**: 467-486.

Frankham, R. 1996. Relationship of genetic variation to population size in wildlife. *Conservation Biology* **10**: 1500-1508.

Galeotti, P. & Cesaris, C. 1996. Rufous and grey colour morphs in the Italian Tawny Owl: Geographical and environmental influences. *Journal of Avian Biology* **27**, 15-20.

Galeotti, P., Rubolini, D., Dunn, P.O. & Fasola, M. 2003 Colour polymorphism in birds: causes and functions. *Journal of Evolutionary biology* **16**, 635-646.

Gehlbach, F.R. 1989. Screech-owl. In: *Lifetime Reproduction in Birds* (I. Newton, ed.), pp. 315-326. Academic Press, London.

Gehlbach, F.R. 1994. *The Eastern Screech-owl: Life History, Ecology and Behaviour in the Suburbs and Countryside.* Texas A & M University Press, College Station, Texas.

GENSTAT, 1993. GENSTAT 5: release 3 reference manual. Oxford: Clarendon Press.

Glutz von Blotzheim, U., Bauer, K. & Bezzel, E. 1971. *Handbuch der Vögel Mitteleuropas.* Aula-Verlag, Wiesbaden.

González, G., Sorci, G. & de Lope, F. 1999. Seasonal variation in the relationship between cellular immune response and badge size in male House Sparrows (*Passer domesticus*). *Behavioral Ecology and Sociobiology* **46**, 117-122.

Greene, E., Lyon, B.E., Muehter, V.R., Ratcliffe, L., Oliver, S.J. & Boag, P. 2000. Disruptive sexual selection for plumage coloration in a passerine bird. *Nature* **407**, 1000-1003.

Gross, M.R. 1996. Alternative reproductive strategies and tactics: diversity within sexes. *Trends in Ecology & Evolution* **11**, 92-98.

Gulland, F. M. D., Albon, S. D., Pemberton, J. M., Moorcroft, P. R. & Clutton-Brock, T. H. 1993. Parasite-associated polymorphism in a cyclic ungulate population. *Proceedings of the Royal Society of London Series B-Biological Sciences* **254**: 7-13.

Hair, J. F., Anderson, R. E., Tatham, R. L. & Black, W. C. 1995. *Multivariate data analysis with readings. 4th ed.* Prentice Hall Int., Englewood Cliffs NJ.

Hänfling, B. & Brandl, R. 1998. Genetic variability, population six=ze and isolation of distinct populations in the freshwater fish *Cottus gobio* L. *Molecular Ecology* **7**: 1625-1632.

Hardy, I.C.W. 2002. *Sex Ratios: Concepts and ratios*. Cambridge University Press.

Harris, M.P., Rothery, P. & Wanlass, S. 2003. Increase in frequency of the bridled morph of the Common Guillemot *Uria aalge* on the Isle of May, 1946-2000: a return to former levels? *Ibis* **145**, 22-29.

Harvey, P. H. & Pagel. M. 1991. *The comparative method in evolutionary biology*. Oxford University Press, Oxford.

Harvey, P. H. & Rambaut, A. 2000. Comparative analyses for adaptive radiations. *Phil. Trans. R. Soc. Lond.* B **355**: 1599-1605.

Hatchwell, B.J., Ross, D.J., Fowlie, M.K. & McGowan, A. 2001. Kin discrimination in cooperatively breeding long-tailed tits. *Proceedings of the Royal Society of London Series B-Biological Sciences* **268**, 885-890.

Hatchwell, B.J., Russell, A.F., Ross, D.J. & Fowlie, M.K. 2000. Divorce in cooperatively breeding long-tailed tits: a consequence of inbreeding avoidance? *Proceedings of the Royal Society of London Series B-Biological Sciences* **267**, 813-819.

Hoffman, E.A. & Blouin, M.S. 2000. A review of colour and pattern polymorphisms in anurans. *Biological Journal of the Linnean Society* **70**, 633-665.

Holdaway, R. N. 1994. An exploratory phylogenetic analysis of the genera of the Accipitridae, with notes on the biogeography of the family. In *Raptor conservation today* (ed. B. U. Meyburg & R. D. Chancellor), pp. 601-647. Berlin: Pica Press.

Horth, L. 2003. Melanic body colour and aggressive mating behaviour are correlated traits in male mosquitofish (Gambusia hotbrooki). *Proceedings of the Royal Society of London Series B-Biological Sciences* **270**, 1033-1040.

Houston, A.I. & McNamara, J.M. 1999. Models of Adaptive Behaviour. An Approach Based on State. Cambridge Univ. Press, Cambridge, U.K.

Hoyt, D. F. 1979. Practical methods of estimating volume and fresh weight of bird eggs. *Auk* **96**: 73-77.

Huxley, J. 1955. Morphism in birds. *Proc. XI. Int. Orn. Congr.*: 309-328.

Itoh, S. 1991. Geographical variation of the plumage polymorphism in the Eastern Reef Heron (*Egretta sacra*). *Condor* **93**, 383-389.

Jacquard, A. 1974. *The genetic structure of populations*. Springer-Verlag, Berlin.

James, F. C. & McCulloch, C. E. 1990. Multivariate analysis in ecology and systematics: Panacea or Pandora´s box? *Ann. Rev. Ecol. Syst.* **21**: 129-166.

Jawor, J.M. & Breitwisch, R. 2003. Melanin ornaments, honesty, and sexual selection. *The Auk* **120**, 249-265.

Jefferies, D.J. & Parslow, J.L.F. 1976. The genetics of bridling in guillemots from a study of hand reared birds. *Journal of Zoology* **179**, 411-420.

Jones, T.M., Quinnell, R.J. & Balmford, A. 1998. Fisherian flies: benefits of female choice in a lekking sandfly. *Proceedings of the Royal Society of London Series B-Biological Sciences* **265**, 1651-1657.

Keller, L.F. 1998. Fitness effects and its fitness effects in an insular population of song sparrows. *Evolution* **52**, 240-250.

Keller, L.F., Arcese, P., Smith J.M., Hochachka, W.M. & Stearns, S. 1994. Selection against inbred song sparrows during a natural population bottleneck. *Nature* **372**, 356-357.

Keller, L.F. & Waller, D.M. 2002. Inbreeding effects in wild populations. *Trends in Ecology and Evolution* **17**, 230-241.

Kirkpatrick, M. & Ryan, M.J. 1991. The evolution of mating preferences and the paradox of the lek. *Nature* **350**, 33-38.

Kokko, H. 1998. Good genes, old age and life-history trade-offs. Evolutionary. Ecololgy. **12**, 739-750.

König, C., Weick, F. & Becking, J. H. 1999. *Owls: a guide to the owls of the world.* Pica Press, East Sussex.

Krüger, O. 2000. Correlates of population density and body weight of raptors in the family Accipitridae: a comparative study. *J. Zool. Lond.* **250**: 185-192.

Krüger, O. 2002. Dissecting common buzzard lifespan and lifetime reproductive success: the relative importance of food, competition, weather, habitat and individual attributes. *Oecologia* **133**, 474-482.

Krüger, O. & Davies, N.B. 2002. The evolution of cuckoo parasitism: a comparative analysis. *Proceedings of the Royal Society of London Series B-Biological Sciences* **269**, 375-381.

Krüger, O. & Lindström, J. 2001. Habitat heterogeneity affects population growth in goshawk *Accipiter gentilis*. *Journal of Animal Ecology* **70**, 173-181.

Krüger, O. & Lindström, J. 2001. Lifetime reproductive success in common buzzard: from individual variation to population demography. *Oikos* **93**, 260-273.

Krüger, O., Lindström, J. & Amos, W. 2001. Maladaptive mate choice maintained by heterozygote advantage. *Evolution* **55**, 1207-1214.

Lacy, R.C., Petric, A. & Warneke, M. 1993. Inbreeding and outbreeding in captive populations of wild animal species. In: *The natural history of inbreeding and outbreedig* (ed Thornhill N.W.), pp. 352-374. University of Chicago Press.

Lank, D.B., Smith, C.M., Hanotte, O., Burke, T. & Cooke, F. 1995. Genetic polymorphism for alternative mating behaviour in lekking male ruff *Philomachus pugnax*. *Nature* **378**, 59-62.

Le Corre, M. 1999. Plumage polymorphism of red-footed boobies (*Sula sula*) in the western Indian Ocean: an indicator of biogeographic isolation. *J. Zool. Lond.* **249**: 411-415.

Losey, J. E., Ives, A. R., Harmon, J., Ballantyne, F. & Brown, C. 1997. A polymorphism maintained by opposite patterns of parasitism and predation. *Nature* **388**: 269-272.

Lowther, J.K. 1961. Polymorphism in the white-throated sparrow, *Zonotrichia*

albicollis (Gmelin). *Canadian Journal of Zoology* **39**, 281-292.

MacColl, A.D.C. & Hatchwell, B.J. 2002. Temporal variation in fitness payoffs promotes cooperative breeding in long-tailed tits Aegithalos caudatus. *American Naturalist* **160**, 186-194.

Madsen, T., Stille, B. & Shine, R. 1996. Inbreeding depression in an isolated population of adders *Vipera berus*. *Biological Conservation* B, 113-118.

Majerus, M.E.N. 1998. *Melanism. Evolution in action.* Oxford University Press.

Mayr, E. 1963. *Animal species and evolution.* Harvard University Press, Cambridge, MA

McNamara, J.M. 1993. State-dependent life-history equations. Acta Biotheoretica 41:165–174.

Melde M. 1983. *Der Mäusebussard. Neue Brehm-Bücherei.* Wittenberg Lutherstadt.

Merilaita, S. & Jormalainen, V. 1997. Evolution of sex differences in microhabitat choice and colour polymorphism in Idotea baltica. *Animal Behaviour* **54**, 769-778.

Merilaita, S. 2001. Habitat heterogeneity, predation and gene flow: Colour polymorphism in the isopod, Idotea baltica. *Evolutionary Ecology* **15**, 103-116.

MINITAB, Release 13 1999. Minitab Inc.

Mosher, J.A. & Henny, C.J. 1976. Thermal adaptiveness of plumage color in screech owls. *Auk* **93**, 614-619.

Murton, R.K. 1971. Polymorphism in Ardeidae. *Ibis* **113**, 97-99.

Murton, R.K., Westwood, N.J. & Thearle, R.J.P. 1973. Polymorphism and the evolution of a continuous breeding season in the pigeon, *Columba livia*. *J.Reprod.Fertil.(Suppl.)* 19, 563-577.

Newton, I. 1979. *Population Ecology of Raptors*. T. & A. D. Poyser, London.

Niecke, M., Rothlaender, S. & Roulin, A. 20003. Why do melanin ornaments signal individual quality? Insights from metal element analysis of barn owl feathers. *Oecologia*. **137**, 153-158.

Nilsson, J.A. & Svensson, E. 1996. The cost of reproduction: A new link between current reproductive effort and future reproductive success. *Proceedings of the Royal Society of London Series B-Biological Sciences* **263**, 711-714.

Nilsson, J.A. 1999 Fitness consequences of timing of reproduction. In: *Proceedings of the XXII international ornithology congress, Durban.* (eds Adams N.J. & Slotow R.H.), pp. 234-247. Birdlife South Africa, Johannesburg, South Africa.

O'Donald, P. 1960. Inbreeding as a result of imprinting. *Heredity* **15**, 79-85.

O'Donald, P. 1983. *The Arctic skua: A study of the ecology and evolution of a seabird.* Cambridge University Press, Cambridge.

Olsson, M. & Shine, R. 1997. The seasonal timing of oviposition in sand lizards (*Lacerta agilis*): Why are early clutches better? *Journal of Evolutionary biology* **10**, 369-381.

Olsson, M., Gullberg, A. & Tegelström, H. 1996. Malformed offspring, sibling matings, and selection against inbreeding in the sand lizard (*Lacerta agilis*). *Journal of Evolutionary biology* **9**, 229-242.

Paulson, D.R. 1973. Predator polymorphism and apostatic selection. *Evolution* **27**, 269-277.

Pemberton, J. M., Albon, S. D., Guinness, F. E. & Clutton-Brock, T. H. 1991. Countervailing selection in different fitness components in female red deer. *Evolution* **45**: 93-103.

Perrins, C.M. 1970. The timing of bird's breeding seasons. *Ibis* **112**, 242-255.

Phillips, R.A. & Furness, R.W. 1998. Polymorphism, mating preferences and sexual selection in the Arctic Skua. *Journal of Zoology* **245**, 245-252.

Pietiainen, H., Saurola, P. & Kolunen, H. 1984. The reproductive constraints on molt in the Ural Owl *Strix uralensis*. *Ann.Zool.Fennici* **21**, 277-281.

Plaistow, S.J. & Tsubaki, Y. 2000. A selective trade-off for territoriality and non-territoriality in the polymorphic damselfly *Mnais costalis*. *Proceedings of the Royal Society of London Series B-Biological Sciences* **267**, 969-975.

Price, T. 1997. Correlated evolution and independent contrasts. *Philosophical Transactions of the Royal Society London.* B **352**: 519-529.

Purvis, A. & Rambaut, A. 1995. Comparative analysis by independent contrasts (CAIC): an Apple Macintosh application for analysing comparative data. *Comp. Appl. Biosci.* **11**: 247-251.

Pusey, A. & Wolf, M. 1996. Inbreeding avoidance in animals. *Trends in Ecology and Evolution* **11**, 201-206.

Pusey, A. 1987. Sex-biased dispersal and inbreeding avoidance in birds and mammals. *Trends in Ecology & Evolution* **2**, 295-299.

Queller, D.C. & Goodnight, K.F. 1989. Estimating relatedness using genetic markers. *Evolution* **43**, 258-275.

Rapoport, E. H. 1982. *Areography: geographical strategies of species*. Pergamon Press, Oxford.

Regan, J.D. 1961. Melanism in the poecillid fish, *Gambusia affinis* (Baird & Girard). *American Midland Naturalist* **65**, 139-143.

Reichle, D. E. 1970. *Analysis of temperate forest ecosystems*. Springer, New York.

Ritland, K., Newton, C. & Marshall, H.D. 2001. Inheritance and population structure of the white-phased "Kermode" black bear. *Current Biology* **11**, 1468-1472.

Rohwer, S. 1983. Formalizing the avoidance-image hypothesis: critique of an earlier prediction. *Auk* **100**, 971-974.

Rohwer, S. & Paulson, D.R. 1987. The avoidance-image hypothesis and colour polymorphism in Buteo hawks. *Ornis Scandinavica* **18**, 285-290.

Saccheri, I., Kuussaari, M., Kankare, M., Vikman, P., Fortelius, W. & Hanski, I. 1998. Inbreeding and extinction in a butterfly metapopulation. *Nature* **392**, 491-494.

Schall, R. 1991. Estimation of generalised linear models with random effects. *Biometrika* **78**: 719-727.

Schönwetter, M. 1967-1992. *Handbuch der Oologie*. Akademie-Verlag, Berlin.

Schultz, E.T. 1993. The effect of birth date on fitness of female dwarf perch, *Micrometus minimus* (Perciformes: Embiotocidae). *Evolution* **47**, 520-539.

Seehausen, O., van Alphen, J. J. M. & Lande, R. 1999. Colour polymorphism and sex ratio distortion in a cichlid fish as an incipient stage in sympatric speciation by sexual selection. *Ecology Letters* **2**: 367-378.

Sinervo, B. & Lively, C.M. 1996. The rock-paper-scissors game and the evolution of alternative male strategies. *Nature* **380**, 240-243.

Singh, S.M. 1981. Polymorphism in colonies of the land snail *Cepaea nemoralis* at London, Ontario - changes over 3 decades. *Canadian Field-Naturalist* **95**, 192-197.

Slate J., L.E.B. Kruuk, T.C. Marshall, J.M. Pemberton and T.H. Clutton-Brock 2000. Inbreeding depression influences lifetime breeding success in a wild population of Red Deer (Cervus elaphus). *Proceedings of the Royal Society of London Series B-Biological Sciences* **267**, 1657-1662.

Soulé, M. 1976. Allozyme variation, its determinants in space and time. In: *Molecular Evolution* (F.J. Ayala, ed.), pp. 60-77. Sinauer Associates, Sunderland, Massaschusetts.

Svensson, E. 1995. Avian reproductive timing – when should parents be prudent. *Animal Behaviour* **49**, 1569-1575.

Svensson, E., Sinervo, B. & Comendant, T. 2001. Density-dependent competition and selection on immune function in genetic lizard morphs. *Proc.Natl.Acad.Sci.USA* **98**, 12561-12565.

Theron, E., Hawkins, K., Bemingham, E., Ricklefs, R.E. & Mundy, N.I. 2001. The molecular basis of an avian plumage polymorphism in the wild: A melanocortin-1-receptor point mutation is perfectly associated with the melanic plumage morph of the bananaquit, *Coereba flaveola*. *Current Biology* **11**, 550-557.

Thiollay, J. M. 1994. Family Accipitridae. In *Handbook of the birds of the world. Vol. 2* (ed. J. del Hoyo, A. Elliott & J. Sargatal), pp. 52-205. Lynx Edicions, Barcelona.

Thorne, M. H., Nicholas, F. W., Moran, C. & Sheldon, B. L. 1997. Genetic analysis of triploidy in a selected line of chickens. *Journal of Heredity* **88**: 495-498.

Thornhill, R. and J. Alcock. 1983. The Evolution of Insect Mating Systems. Harvard University Press, Cambridge, Massachusetts.

Thornycroft, H.B. 1975. A cytogenetic study of the white-throated sparrow *Zonotrichia albicollis*. *Evolution* **29**, 611-621.

Tsubaki, Y., Hooper, R.E. & Siva-Jothy, M.T. 1997. Differences in adult and reproductive lifespan in the two male forms *Mnais pruinosa costalis* (Odonata: Caopterygidae). *Res.Popul, Ecol.* **39**, 149-155.

Tuttle, E.M. 2003. Alternative reproductive strategies in the white-throated sparrow: behavioral and genetic evidence. *Behavioral Ecology* **14**, 425-432.

West P.M. and C. Packer. 2002. Sexual selection, temperature, and the Lion's mane. Science. **297**: 1339-1343.

Wink, M. & Heidrich, P. 1999. Molecular evolution and systematics of owls (Strigiformes). In *Owls: a guide to the owls of the world.* (ed. C. König, F. Weick & J. H. Becking), pp. 39-57. Pica Press, East Sussex.

Wink, M. & Sauer-Gürth, H. 2000. Advances in the molecular systematics of African raptors. In *Raptors at risk.* (ed. R. D. Chancellor & B. U. Meyburg), pp. 135-147. Hancock House Publishers, Surrey BC.

Witschi, E. & Miller, R.A. 1938. Ambisexuality in the female starling. *Journal of Experimental Zoology* **79**, 475-487.

Zang, H., Heckenroth, H. & Knolle, F. 1989. Die Vögel Niedersachsens-Greifvögel. Naturschutz Landschaftspfl. *Niedersachs.B, H* 2.3.